

Stucker 09/980,777

=> d que 11
L1

0 SEA FILE=REGISTRY IMTGDT PINIFGRNI[-L] TALGMSLNLPVAKV/SQSP

=> d his 1

(FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
14:18:43 ON 25 SEP 2003)

L17 51 DUP REM L16 (35 DUPLICATES REMOVED)

=> d que 117

L2 114 SEA TELLES J?/AU
L3 343 SEA DESCAMPS D?/AU
L4 654 SEA ("BRUN VESINET C"/AU OR "BRUN VESINET F"/AU OR "BRUN
VESINET FRANCOISE"/AU OR "BRUN VEZINET"/AU OR "BRUN VEZINET
B"/AU OR "BRUN VEZINET C"/AU OR "BRUN VEZINET F"/AU OR "BRUN
VEZINET F B"/AU OR "BRUN VEZINET F C"/AU OR "BRUN VEZINET
FR"/AU OR "BRUN VEZINET FRAN CEDILLA OISE"/AU OR "BRUN VEZINET
FRANCOISE"/AU OR "BRUN VEZINET V"/AU OR "BRUN VEZINT F"/AU OR
"BRUN VEZIRET F"/AU)
L5 165 SEA ("BRUNVESINET C"/AU OR "BRUNVESINET F"/AU OR "BRUNVEZINET
C"/AU OR "BRUNVEZINET F"/AU)
L6 1076 SEA (L2 OR L3 OR L4 OR L5)
L7 158 SEA L6 AND (HIV-2 OR HIV(A) 2 OR HIV2 OR HIVII)
L8 11 SEA L7 AND PROTEASE#
L9 415 SEA (HIV-2 OR HIV(A) 2 OR HIV2 OR HIVII OR HIV-II OR HIV(A)
II) (5A) PROTEASE#
L10 4831 SEA (HUMAN(3A) IMMUNODEFICIENCY(A) VIRUS) (A) (2 OR II)
L11 584 SEA ((L9 OR L10)) AND PROTEASE#
L12 589 SEA L8 OR L11
L13 109546 SEA PROTEASE#(3A) INHIBIT?
L14 372 SEA L12 AND L13
L15 83 SEA L14 AND RESISTAN?
L16 86 SEA L8 OR L15
L17 51 DUP REM L16 (35 DUPLICATES REMOVED)

=> d ibib abs 117 1-51

L17 ANSWER 1 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2003:118034 HCAPLUS

DOCUMENT NUMBER: 138:163486

TITLE: **protease** autocatalytic precursor-reporter
protein fusion product for **protease**

inhibitor identification and drug screening
INVENTOR(S): Konvalinka, Jan; Uhlikova, Tat'ana; Dantuma, Nicolaas
Pieter; Masucci, Maria; Lindsten, Kristina

PATENT ASSIGNEE(S): Ustav Organické Chemie a Biochemie Akademie Ved Ceske
Republiky, Czech Rep.; Karolinska Innovations AB

SOURCE: PCT Int. Appl., 43 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|------|----------|-----------------|----------|
| WO 2003012131 | A2 | 20030213 | WO 2002-EP8142 | 20020722 |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AA, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-308312P P 20010727

AB A fusion protein is provided in cells in culture that comprises (i) an autoproteolytic precursor of a cytotoxic **protease** and (ii) a reporter protein, which reporter protein provides a quant. signal indicative of amt. of reporter protein present within the cells, allowing for assays to identify **inhibitors** of the **protease** and potential therapeutics useful in targeting pathogens, esp. viruses such as HIV.

L17 ANSWER 2 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2003:23919 HCAPLUS

DOCUMENT NUMBER: 139:368

TITLE: Actinomycin D induces high-level **resistance** to thymidine analogs in replication of human immunodeficiency virus type 1 by interfering with host cell thymidine kinase expression

AUTHOR(S): Imamichi, Tomozumi; Murphy, Michael A.; Adelsberger, Joseph W.; Yang, Jun; Watkins, Catherine M.; Berg, Steve C.; Baseler, Michael W.; Lempicki, Richard A.; Guo, Jianhui; Levin, Judith G.; Lane, H. Clifford

CORPORATE SOURCE: Laboratory of Molecular Retrovirology, Clinical Services Program, National Cancer Institute-Frederick, Science Applications International Corporation-Frederick Inc., Frederick, MD, 21702, USA

SOURCE: Journal of Virology (2003), 77(2), 1011-1020

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Actinomycin D (ActD) is a transcription inhibitor and has been used in the treatment of certain forms of cancer. ActD has been reported to be a potential inhibitor of human immunodeficiency virus type 1 (HIV-1) replication due to its ability to inhibit reverse transcription. In contrast to what was expected, low concns. of ActD (1 to 10 nM) upregulated HIV-1 replication 8- to 10-fold in MT-2 cells and had no effect on HIV-2 replication or on HIV-1 replication in MT-4, Jurkat, or peripheral blood mononuclear cells. The upregulation of HIV-1 replication was assocd. with an increase in HIV-1 transcription and a decrease in CD4 and CXCR4 expression. To further evaluate the effects of ActD on emergence of drug **resistance** in HIV-1 replication, a series of drug **resistance** assays were performed. Of interest, treatment of MT-2 cells with ActD also led to a high level of **resistance** to thymidine analogs (>1,000-fold increase in **resistance** to zidovudine and >250-fold to stavudine) but not to other nucleoside reverse transcriptases (RT), nonnucleoside RT, or **protease inhibitors**. This **resistance** appeared to be due to a suppression of host cell thymidine kinase-1 (TK-1) expression. These results indicate that ActD leads to a novel form of thymidine analog

resistance by suppressing host cell TK-1 expression. These results suggest that administration of combination drugs to HIV-1-infected patients may induce **resistance** to antiretroviral compds. via a modification of cellular factors.

REFERENCE COUNT: 63 THERE ARE 63 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 3 OF 51 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:400601 BIOSIS

DOCUMENT NUMBER: PREV200300400601

TITLE: Clinical, immunological and virological response to different antiretroviral regimens in a cohort of HIV-2-infected patients.

AUTHOR(S): van der Ende, Marchina E. (1); Prins, Jan M.; Brinkman, Kees; Keuter, Monique; Veenstra, Jan; Danner, Sven A.; Niesters, Hubert G. M.; Osterhaus, Albert D. M. E.; Schutten, Martin

CORPORATE SOURCE: (1) University Hospital Rotterdam, Dijkzigt, Dr Molewaterplein 40, 3015 GD, Rotterdam, Netherlands: vanderende@inw2.azr.nl Netherlands

SOURCE: AIDS (Hagerstown), (July 2003, 2003) Vol. 17, No. Supplement 3, pp. S55-S61. print. ISSN: 0269-9370.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Objective: To assess the clinical, immunological and virological response and the emergence of **resistance** towards antiretroviral therapy (ART) in a cohort of HIV-2-infected patients. Design: Observational study. Patients: HIV-2-infected patients residing in the Netherlands. Results: From 1995 to 2001 seven patients failed various ART regimens. The **resistance** mutations were analysed retrospectively. Development of mutations proved to be similar to that observed in HIV-1-infected patients, with the exception of a higher occurrence of the Q151M mutation within the reverse transcriptase gene. In a prospective study, comprising 13 consecutive naive HIV-2-infected patients, all patients achieved plasma HIV-2-RNA suppression below the detection limit (500 copies/ml). The antiretroviral regimen consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) and indinavir, with a boosting dose of ritonavir; the median follow-up was 91 weeks. Two patients experienced a temporary virological rebound, while at the same time therapeutic drug monitoring showed sub-therapeutic plasma levels of indinavir. Conclusion: Sustained viral suppression in HIV-2-infected patients can be achieved using an antiretroviral regimen of two NRTIs and boosted indinavir or lopinavir.

L17 ANSWER 4 OF 51 MEDLINE on STN

ACCESSION NUMBER: 2003280496 IN-PROCESS

DOCUMENT NUMBER: 22691954 PubMed ID: 12807687

TITLE: Diagnosis, antiretroviral therapy, and emergence of **resistance** to antiretroviral agents in HIV-2 infection: a review.

AUTHOR: Hightower Maia; Kallas Esper Georges

CORPORATE SOURCE: Infectious Diseases Discipline, Federal University of Sao Paulo, Sao Paulo, SP, Brazil.

SOURCE: BRAZILIAN JOURNAL OF INFECTIOUS DISEASES, (2003 Feb) 7 (1) 7-15.

Journal code: 9812937. ISSN: 1413-8670.

PUB. COUNTRY: Brazil

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

Stucker 09/980,777

FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20030617
Last Updated on STN: 20030801

AB Human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) are the causative agents of AIDS. HIV-2 is prevalent at moderate to high rates in West African countries, such as Senegal, Guinea, Gambia, and Cape Verde. Diagnosis of HIV-2 is made with a positive HIV-1/HIV-2 ELISA or simple/rapid assay, followed by one or two confirmatory tests specific for HIV-2. Following CD(4)(+)T cell counts, HIV-2 viral burden and clinical signs and symptoms of immunodeficiency are beneficial in monitoring HIV-2 disease progression. Although non-nucleoside reverse transcriptase inhibitors are ineffective in treating HIV-2, nucleoside reverse transcriptase inhibitors and protease inhibitors can be effective in dual and triple antiretroviral regimens. Their use can decrease HIV-2 viral load, increase CD(4)(+)T cell counts and improve AIDS-related symptoms. HIV-2 resistance to various nucleoside reverse transcriptase inhibitors and protease inhibitors, including zidovudine, lamivudine, ritonavir and indinavir, has been identified in some HIV-2 infected patients on antiretroviral therapy. The knowledge of HIV-2 peculiarities, when compared to HIV-1, is crucial to helping diagnose and guide the clinician in the choice of the initial antiretroviral regimen and for monitoring therapy success.

L17 ANSWER 5 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:888736 HCAPLUS

DOCUMENT NUMBER: 137:384835

TITLE: Preparation of 2-amino-benzoxazole sulfonamide as broad-spectrum HIV protease inhibitors

INVENTOR(S): Surleraux, Dominique Louis Nestor Ghislain; Vendeville, Sandrine Marie Helene; Verschueren, Wim Gaston; De Bethune, Marie-Pierre T. M. M. G.; De Kock, Herman Augustinus; Tahri, Abdellah

PATENT ASSIGNEE(S): Tibotec Pharmaceuticals Ltd., Ire.

SOURCE: PCT Int. Appl., 54 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

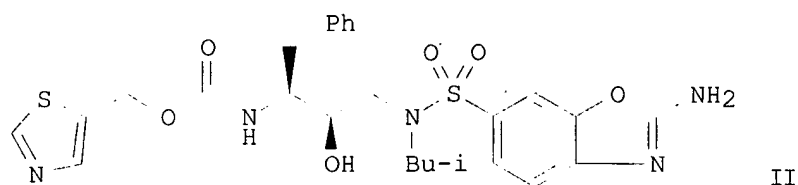
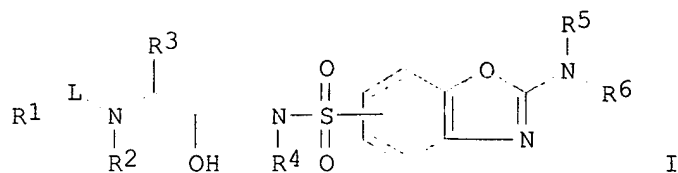
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|--|----------|-----------------|----------|
| WO 2002092595 | A1 | 20021121 | WO 2002-EP5212 | 20020510 |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | |

PRIORITY APPLN. INFO.: EP 2001-201732 A 20010511

OTHER SOURCE(S): MARPAT 137:384835

GI



AB Title compds. I [R1, R8 = H, alkyl, alkenyl, arylalkyl, cycloalkyl, aryl, heterocyclyl, etc.; R2 = H, alkyl; L = CO, OCO, NR8CO, etc.; R3 = alkyl, cycloalkyl, aryl, etc.; R4 = H, alkoxy carbonyl, carboxy, aminocarbonyl, cycloalkyl, etc.; R5-6 = H, alkyl], N-oxides, stereoisomers, metabolites and prodrugs thereof were prep'd. For instance, II was prep'd. from the corresponding diamine (prepn. described), N,N'-disuccinimidylcarbonate and 5-hydroxymethylthiazole (CH₂Cl₂, 6 h). Compds. of the invention are effective in inhibiting a broad range of mutant HIV strains; II had pEC50 = 8.18 against HIV-1 (Lai strain).

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 6 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:51250 HCAPLUS

DOCUMENT NUMBER: 136:96025

TITLE: Pharmaceutical composition and method for the treatment of retroviral infections

INVENTOR(S): De Clercq, Erik; Debyser, Zeger; Plymers, Willem;
Pannecouque, Christophe; Witvrouw, Myriam

PATENT ASSIGNEE(S): Stichting Rega Vzw, Belg.

SOURCE: PCT Int. Appl., 45 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

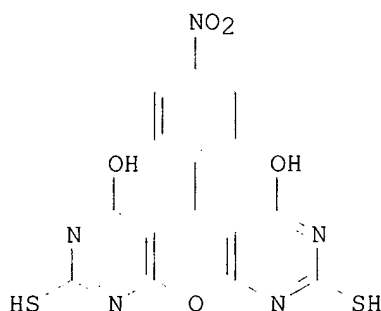
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|------|----------|-----------------|----------|
| WO 2002003971 | A2 | 20020117 | WO 2001-EP7882 | 20010709 |
| WO 2002003971 | A3 | 20020516 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT,

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RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US,
UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
EP 1309349 A2 20030514 EP 2001-956518 20010709
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
PRIORITY APPLN. INFO.: GB 2000-16845 A 20000707
US 2000-234416P P 20000921
US 2001-262612P P 20010118
WO 2001-EP7882 W 20010709
OTHER SOURCE(S): MARPAT 136:96025
GI



AB The invention provides the use of a pyrano[2,3-d:6,5-d'] dipyrimidine deriv. for the manuf. of a medicine for the treatment of a retroviral infection in a mammal. It also provides a product contg.: (a) one or more derivs. of a pyrano[2,3-d:6,5-d'] dipyrimidine; and (b) one or more anti-retroviral drugs, including drugs effective against one or more retroviral or cellular proteins involved in the entry and/or replication of a retrovirus, in resp. proportions such as to provide a synergistic effect against a retroviral infection in a mammal, as a combined prepn. for simultaneous, sep. or sequential use in retroviral infection therapy. Examples demonstrating the efficacy of several pyranodipyrimidines against HIV-1, HIV-2, SIV, and drug-resistant HIV-1 strains are provided. Synergistic effects of I may be achieved when used in combination with other drugs such as zidovudine, nevirapine and nelfinavir.

L17 ANSWER 7 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:488245 HCAPLUS

DOCUMENT NUMBER: 137:57593

TITLE: Compositions and methods using alkyl- and phospholipid-drug conjugates for double-targeting virus infections and cancer cells

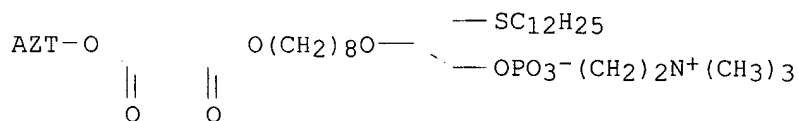
INVENTOR(S): Kucera, Louis S.; Fleming, Ronald A.; Ishaq, Khalid S.; Kucera, Gregory L.; Morris-Natschke, Susan L.

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 35 pp., Cont.-in-part of U.S. Ser. No. 693,658.

| | |
|-------------------------|---------------|
| DOCUMENT TYPE: | CODEN: USXXCO |
| LANGUAGE: | Patent |
| FAMILY ACC. NUM. COUNT: | English |
| PATENT INFORMATION: | 1 |

| | | | | |
|------------------------|------------------|----------|-----------------|-------------|
| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
| ----- | ---- | ----- | ----- | ----- |
| US 2002082242 | A1 | 20020627 | US 2001-844201 | 20010427 |
| PRIORITY APPLN. INFO.: | | | US 2000-693658 | A2 20001019 |
| OTHER SOURCE(S): | MARPAT 137:57593 | | | |
| GI | | | | |



AB The invention includes compns. and methods useful for treatment of a virus infection in a mammal by double-targeting the virus (i.e. targeting the virus at more than one stage of the virus life cycle) and thereby inhibiting virus replication. The compns. of the invention include compds., which comprise a phosphocholine moiety covalently conjugated with one or more therapeutic agents (e.g. nucleoside analog, **protease inhibitor**, etc.) to a lipid backbone. The invention also includes pharmaceutical compns. for use in treatment of a virus infection in mammals. The methods of the invention comprise administering a compd. of the invention, a pharmaceutically acceptable salt or a prodrug thereof, or a pharmaceutical compn. of the invention, in an amt. effective to treat the infection, to a mammal infected with a virus. Addnl., the invention includes compns. and methods useful for combating a cancer in a mammal and facilitating delivery of a therapeutic agent to a mammalian cell. The compns. of the invention include compds., which comprise an alkyl lipid or phospholipid moiety covalently conjugated with a therapeutic agent (e.g., a nucleoside analog). The invention also includes pharmaceutical compns. for combating cancer and facilitating delivery of a therapeutic agent to a mammalian cell. The methods of the invention comprise administering a compd. of the invention, a pharmaceutically acceptable salt or a prodrug thereof, or a pharmaceutical compn. of the invention, in an amt. effective to combat a cancer or to facilitate delivery of a therapeutic agent to a mammalian cell. Prepn. of INK-20 (I) is described.

L17 ANSWER 8 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:79486 HCAPLUS
DOCUMENT NUMBER: 136:363282
TITLE: A potent human immunodeficiency virus type 1
protease inhibitor, UIC-94003
(TMC-126), and selection of a novel (A28S) mutation in
the **protease** active site
AUTHOR(S): Yoshimura, Kazuhisa; Kato, Ryohei; Kavlick, Mark F.;
Nguyen, Aline; Maroun, Victor; Maeda, Kenji; Hussain,
Khaja A.; Ghosh, Arun K.; Gulnik, Sergei V.; Erickson,
John W.; Mitsuya, Hiroaki
CORPORATE SOURCE: Experimental Retrovirology Section, Medicine Branch,
Division of Clinical Sciences, National Cancer
Institute, National Institutes of Health, Bethesda,

MD, 20822, USA
SOURCE: Journal of Virology (2002), 76(3), 1349-1358
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We identified UIC-94003, a nonpeptidic human immunodeficiency virus (HIV) **protease inhibitor** (PI), contg. 3(R),3a(S),6a(R)-bis-tetrahydrofuranyl urethane (bis-THF) and a sulfonamide isostere, which is extremely potent against a wide spectrum of HIV (50% inhibitory concn., 0.0003 to 0.0005 .mu.M). UIC-94003 was also potent against multi-PI-**resistant** HIV-1 strains isolated from patients who had no response to any existing antiviral regimens after having received a variety of antiviral agents (50% inhibitory concn., 0.0005 to 0.0055 .mu.M). Upon selection of HIV-1 in the presence of UIC-94003, mutants carrying a novel active-site mutation, A28S, in the presence of L10F, M46I, I50V, A71V, and N88D appeared. Modeling anal. revealed that the close contact of UIC-94003 with the main chains of the **protease** active-site amino acids (Asp29 and Asp30) differed from that of other PIs and may be important for its potency and wide-spectrum activity against a variety of drug-**resistant** HIV-1 variants. Thus, introduction of inhibitor interactions with the main chains of key amino acids and seeking a unique inhibitor-enzyme contact profile should provide a framework for developing novel PIs for treating patients harboring multi-PI-**resistant** HIV-1.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 9 OF 51 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:468629 BIOSIS
DOCUMENT NUMBER: PREV200200468629
TITLE: New class of HIV integrase inhibitors that block viral replication in cell culture.
AUTHOR(S): Pannecouque, Christophe; Pluymers, Wim; Van Maele, Benedicte; Tetz, Victor; Cherepanov, Peter; De Clercq, Erik; Witvrouw, Myriam; Debyser, Zeger (1)
CORPORATE SOURCE: (1) Rega Institute for Medical Research, KULeuven, Minderbroedersstraat 10, B-3000, Leuven: zeger.debyser@uz.kuleuven.ac.be Belgium
SOURCE: Current Biology, (July 23, 2002) Vol. 12, No. 14, pp. 1169-1177. <http://www.current-biology.com/>. print. ISSN: 0960-9822.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Background: To improve the existing combination therapies of infection with the human immunodeficiency virus (HIV) and to cope with virus strains that are **resistant** to multiple drugs, we initiated a search for effective inhibitors of HIV integrase, the enzyme responsible for inserting the viral cDNA into the host cell chromosome. Results: We have now identified a series of 5H-pyrano(2,3-d:-6,5-d')dipyrimidines that block the replication of various strains of HIV-1 and HIV-2. The most potent congener, 5-(4-nitrophenyl)-2,8-dithiol-4,6-dihydroxy-5H-pyrano(2,3-d:-6,5-d')dipyrimidine (V-165), inhibited the replication of HIV-1(IIIB) in MT-4 cells at a 50% effective concentration (EC50) of 8.9 muM, which is 14-fold below its cytotoxic concentration. V-165 was equally active against virus strains that were **resistant** toward inhibitors of viral entry or reverse transcriptase. In combination regimens in cell culture, V-165 acted subsynergistically with zidovudine or nelfinavir and synergistically with nevirapine. V-165 inhibited both reverse

transcriptase and integrase activities in enzymatic assays at micromolar concentrations, but only a close correlation was found between the anti-HIV activity observed in cell culture and the inhibitory activity in the integrase strand transfer assays. Time-of-addition experiments indicated that V-165 interfered with the viral replication cycle at a time point coinciding with integration. Quantitative Alu-PCR corroborated that the anti-HIV activity of V-165 is based upon the inhibition of proviral DNA integration. Conclusions: Based on their mode of action, which is different from that of clinically approved anti-HIV drugs, PDPs are good candidates for further development into new drugs and to be included in future combination regimens.

L17 ANSWER 10 OF 51 MEDLINE on STN DUPLICATE 1
 ACCESSION NUMBER: 2002211615 MEDLINE
 DOCUMENT NUMBER: 21942111 PubMed ID: 11944889
 TITLE: HIV-1 Vif-derived peptide **inhibits** drug-resistant HIV **proteases**.
 AUTHOR: Blumenzweig Immanuel; Baraz Lea; Friedler Assaf; Danielson U Helena; Gilon Chaim; Steinitz Michael; Kotler Moshe
 CORPORATE SOURCE: Department of Pathology, Hebrew University-Hadassah Medical School, Jerusalem, 91120, Israel.
 SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2002 Apr 12) 292 (4) 832-40.
 Journal code: 0372516. ISSN: 0006-291X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200205
 ENTRY DATE: Entered STN: 20020412
 Last Updated on STN: 20020514
 Entered Medline: 20020513

AB Vif, one of the six accessory genes expressed by HIV-1, is essential for the productive infection of natural target cells. Previously we suggested that Vif acts as a regulator of the viral **protease** (PR): It prevents the autoprocessing of Gag and Gag-Pol precursors until virus assembly, and it may control the PR activity in the preintegration complex at the early stage of infection. It was demonstrated before that Vif, and specifically the 98 amino acid stretch residing at the N'-terminal part of Vif (N'-Vif), inhibits both the autoprocessing of truncated Gag-Pol polyproteins in bacterial cells and the hydrolysis of synthetic peptides by PR in cell-free systems. Linear synthetic peptides derived from N'-Vif specifically inhibit and bind HIV-1 PR in vitro, and arrest virus production in tissue culture. Peptide mapping of N'-Vif revealed that Vif88-98 is the most potent PR inhibitor. Here we report that this peptide inhibits both HIV-1 and **HIV-2**, but not ASLV **proteases** in vitro. Vif88-98 retains its inhibitory effect against drug-resistant HIV-1 PR variants, isolated from patients undergoing long-term treatment with anti-PR drugs. Variants of HIV **protease** bearing the mutation G48V are **resistant** to inhibition by this Vif-derived peptide, as shown by in vitro assays. In agreement with the in vitro experiments, Vif88-98 has no effect on the production of infectious particles in cells infected with a G48V mutated virus.
 (c)2002 Elsevier Science (USA).

L17 ANSWER 11 OF 51 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
 on STN
 ACCESSION NUMBER: 2003017046 EMBASE

TITLE: Unusual binding mode of an HIV-1 **protease inhibitor** explains its potency against multi-drug-resistant virus strains.

AUTHOR: Weber J.; Mesters J.R.; Lepsik M.; Prejdova J.; Svec M.; Sponarova J.; Mlcochova P.; Skalicka K.; Strisovsky K.; Uhlikova T.; Soucek M.; Machala L.; Stankova M.; Vondrasek J.; Klimkait T.; Kraeusslich H.-G.; Hilgenfeld R.; Konvalinka J.

CORPORATE SOURCE: J. Konvalinka, Inst. of Organic Chemistry/Biochem., Acad. of Sci. of the Czech Republic, Protease of Human Pathogens, Flemingovo n. 2, CZ-16610 Praha 6, Czech Republic. konval@uochb.cas.cz

SOURCE: Journal of Molecular Biology, (2002) 324/4 (739-754). Refs: 55
ISSN: 0022-2836 CODEN: JMOBAK

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
030 Pharmacology
037 Drug Literature Index

LANGUAGE: English

SUMMARY LANGUAGE: English

AB **Protease inhibitors** (PIs) are an important class of drugs for the treatment of HIV infection. However, in the course of treatment, **resistant** viral variants with reduced sensitivity to PIs often emerge and become a major obstacle to successful control of viral load. On the basis of a compound equipotently inhibiting HIV-1 and 2 **proteases** (PR), we have designed a pseudopeptide inhibitor, QF34, that efficiently inhibits a wide variety of PR variants. In order to analyze the potency of the inhibitor, we constructed PR species harboring the typical (signature) mutations that confer **resistance** to commercially available PIs. Kinetic analyses showed that these mutated PRs were inhibited up to 1000-fold less efficiently by the clinically approved PIs. In contrast, all PR species were effectively inhibited by QF34. In a clinical study, we have monitored 30 HIV-positive patients in the Czech Republic undergoing highly active antiretroviral therapy, and have identified highly PI **resistant** variants. Kinetic analyses revealed that QF34 retained its subnanomolar potency against multi-drug **resistant** PR variants. X-ray crystallographic analysis and molecular modeling experiments explained the wide specificity of QF34: this inhibitor binds to the PR in an unusual manner, thus avoiding contact sites that are mutated upon **resistance** development, and the unusual binding mode and consequently the binding energy is therefore preserved in the complex with a **resistant** variant. These results suggest a promising route for the design of second-generation PIs that are active against a variety of **resistant** PR variants. .COPYRGHT. 2002 Elsevier Science Ltd. All rights reserved.

L17 ANSWER 12 OF 51 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:347821 BIOSIS

DOCUMENT NUMBER: PREV200300347821

TITLE: A novel HIV inhibitor that targets the nucleocapsid protein.

AUTHOR(S): Hou, X. (1); Zhao, Y. (1); Fabrycki, J. (1); Liu, D. (1); Marlbor, C. (1); Thoma, C. (1); Yang, W. (1); Song, Y. (1); Bradbury, B. (1); Rice, W. (1); Deshpande, M. (1); Huang, M. (1); Buechter, D. (1)

CORPORATE SOURCE: (1) Achillion Pharmaceuticals, New Haven, CT, USA USA

SOURCE: Abstracts of the Interscience Conference on Antimicrobial

Agents and Chemotherapy, (2002) Vol. 42, pp. 266. print.
Meeting Info.: 42nd Interscience Conference on
Antimicrobial Agents and Chemotherapy San Diego, CA, USA
September 27-30, 2002 American Society for Microbiology

DOCUMENT TYPE: Conference
LANGUAGE: English

AB Background: Multi-drug therapy of AIDS has delayed the onset of clinical disease and death but there continues to be a need for drugs against novel targets that are efficacious, non-toxic, and less prone to the development of **resistance**. The nucleocapsid protein (NC) of HIV-1 contains two zinc fingers that are attractive as antiviral targets because they are novel, essential for replication and infectivity, and highly conserved. Previous work described the in vitro anti-HIV activity profiles of novel NC inhibitors. Here we expand on this work and present studies of a representative lead compound of a chemotype that targets HIV-1 NC. Methods: Antiviral activity was measured in a cytoprotection assay; enzymatic or binding assays were used for zinc finger-containing proteins; cytotoxicity was measured by MTS reduction. Results: Compound ACH100,703 has in vitro activity against HIV-1 (including clinical isolates) and HIV-2 with a therapeutic index >100. It is active against viruses **resistant to protease** and reverse transcriptase **inhibitors**. Members of the ACH100,703 chemotype are additive or synergistic with other antiviral agents, including nucleoside and nonnucleoside reverse transcriptase **inhibitors** and **protease inhibitors**. ACH100,703 retains activity in the presence of serum proteins and is stable in vitro in human plasma. ACH100,703 exhibits specificity for the NC protein and does not inhibit the activity of tested mammalian proteins containing a variety of zinc finger prototypes. ACH100,703 shows minimal toxicity in a variety of mammalian cell lines. Studies to determine additional safety and PK parameters in both in vitro and in vivo models are in progress. Conclusions: ACH100,703 and related compounds are highly specific, non-cytotoxic novel anti-HIV compounds that may be clinically useful agents with suitable PK properties and attractive safety and toxicity profiles. Further development of this chemotype as clinical candidates is in progress.

L17 ANSWER 13 OF 51 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

ACCESSION NUMBER: 2002:679485 SCISEARCH

THE GENUINE ARTICLE: 583HM

TITLE: Selection of mutations in **HIV-2**
protease gene in patients receiving first line
protease inhibitors containing regimen

AUTHOR: **Descamps D (Reprint)**; Damond F; Matheron S;
Farfara I; Lastere S; Campa P; Foiny P; Pueyo S; Chene G;
Brun-Vezinet F

CORPORATE SOURCE: Hop Bichat Claude Bernard, F-75677 Paris 18, France;
INSERM, U330, Bordeaux, France

COUNTRY OF AUTHOR: France

SOURCE: ANTIVIRAL THERAPY, (JUN 2002) Vol. 7, Supp. [1], pp.
S150-S150. MA 138.
Publisher: INT MEDICAL PRESS LTD, 2-4 IDOL LANE, LONDON
EC3R 5DD, ENGLAND.
ISSN: 1359-6535.

DOCUMENT TYPE: Conference; Journal

LANGUAGE: English

REFERENCE COUNT: 0

L17 ANSWER 14 OF 51 MEDLINE on STN DUPLICATE 2
 ACCESSION NUMBER: 2002657091 MEDLINE
 DOCUMENT NUMBER: 22304426 PubMed ID: 12416447
 TITLE: NNRTI plus PI combinations in the perspective of
 nucleoside-sparing or nucleoside-failing antiretroviral
 regimens.
 AUTHOR: Joly Veronique; **Descamps Diane**; Yeni Patrick
 CORPORATE SOURCE: Hopital Bichat-Claude Bernard 16 rue Henri Huchard 75877
 Paris, France.. veronique.joly@bch.ap-hop-paris.fr
 SOURCE: AIDS Rev, (2002 Jul-Sep) 4 (3) 128-39. Ref: 86
 Journal code: 101134876. ISSN: 1139-6121.
 PUB. COUNTRY: Spain
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 (REVIEW, TUTORIAL)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200301
 ENTRY DATE: Entered STN: 20021106
 Last Updated on STN: 20030111
 Entered Medline: 20030110

AB Although not yet recommended, regimens combining both a non-nucleoside
 reverse transcriptase **inhibitor** (NNRTI) and **protease**
inhibitors (PI) can be used as first-line therapy, or as
 second-line or salvage therapy in patients who need to change
 antiretroviral treatment because of nucleoside reverse transcriptase
 inhibitors (NRTI) intolerance or virological failure with
resistance to NRTI. Such combinations should not be used in
 patients infected with HIV-1 group O and HIV-2, due to
 the natural **resistance** to NNRTI of these subtypes. Dual NNRTI
 and PI combinations used as first-line therapy allow to spare NRTI,
 leaving a fully active class of drugs for later use, and delaying the risk
 of toxicity related to NRTI exposure, particularly mitochondrial toxicity.
 Several studies have shown that adding a NNRTI improves the efficacy of a
 second-line or salvage therapy based on a new combination of PI(s) and new
 or recycled NRTI(s). A possible explanation for the efficacy of
 NNRTI-containing regimens in NRTI-pretreated patients is that mutations
 conferring **resistance** to NRTI can increase the susceptibility of
 the viruses to the NNRTI. However, the decision to use a NNRTI in a
 salvage regimen needs to be weighed against the concern that subsequent
 failure will exhaust therapeutic options with any compound of this class,
 due the large degree of cross-**resistance** between the three
 available NNRTI. NNRTI and PIs are extensively metabolized in the liver
 through cytochrome P450, leading to pharmacokinetic interactions. The
 decrease in PIs plasma concentrations observed when they are combined with
 nevirapine or efavirenz is reduced when low doses of ritonavir, which
 strongly inhibits cytochrome P450, are associated with the combination of
 PI and NNRTI.

L17 ANSWER 15 OF 51 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 ACCESSION NUMBER: 2002:631606 BIOSIS
 DOCUMENT NUMBER: PREV200200631606
 TITLE: Structure-based design of AIDS drugs and the development of
resistance.
 AUTHOR(S): Wlodawer, Alexander (1)
 CORPORATE SOURCE: (1) Macromolecular Crystallography Laboratory, National
 Cancer Institute at Frederick, Frederick, MD:
 wlodawer@ncifcrf.gov USA
 SOURCE: Vox Sanguinis, (August, 2002) Vol. 83, No. Supplement 1,

pp. 023-026. <http://www.blackwell-science.com/vox>. print.
ISSN: 0042-9007.

DOCUMENT TYPE: General Review
LANGUAGE: English

L17 ANSWER 16 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2001:816971 HCAPLUS

DOCUMENT NUMBER: 135:353725

TITLE: Method for detection of minority genomes in virus
quasispecies using DNA microchips

INVENTOR(S): Arias Esteban, Armando; Baranowski, Eric; Briones
Llorente, Carlos; Domingo Solans, Esteban; Escarmis
Homs, Cristina; Gomez Castilla, Jordi; Martin
Ruiz-Jarabo, Carmen; Parro Garcia, Victor

PATENT ASSIGNEE(S): Consejo Superior De Investigaciones Cientificas,
Spain; Parro Garcia, Victor

SOURCE: PCT Int. Appl., 107 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Spanish

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|------------|
| WO 2001083815 | A1 | 20011108 | WO 2001-ES165 | 20010427 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| EP 1284296 | A1 | 20030219 | EP 2001-929654 | 20010427 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| PRIORITY APPLN. INFO.: | | | ES 2000-1068 | A 20000427 |
| | | | WO 2001-ES165 | W 20010427 |

AB The invention relates to a method for the detection of minority genomes in virus quasispecies using DNA microchips. The method makes it possible to detect, in a nucleic acids population of a virus quasispecies, minority genomes, more particularly minority memory genomes comprising <50% of a population and contg. at least one mutation relative to the majority genomes of said quasispecies. Said method involves the following steps: (a) extg. the nucleic acid of said virus quasispecies from a sample suspected of contg. said virus quasispecies; (b) amplifying at least one fragment of the nucleic acid of said virus quasispecies; and (c) detecting and analyzing the existence of minority genomes using DNA microchip-based techniques. The method can be used in genetic diagnosis of viral diseases. Thus, based on a list of mutations in genes for HIV-1 reverse transcriptase- and **protease-inhibiting** drugs, a DNA microarray for detection of these mutations was prepd. This microarray was used to analyze HIV mutations in an AIDS patient treated with AZT, 3TC, and Indinavir. Mutations were again assessed after further treatment with ddI, 2',3'-Didehydro-2',3'-dideoxythymidine, and Indinavir. Mutations assocd. with "memory genomes" were clearly observable.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 17 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
 ACCESSION NUMBER: 2001:581691 HCAPLUS
 DOCUMENT NUMBER: 135:162484
 TITLE: Aromatic guanylhydrazones and their therapeutic use,
 especially for prophylaxis and treatment of
 bacterially or virally caused diseases and infections
 INVENTOR(S): Bevec, Dorian; Hauber, Joachim; Obert, Sabine; Keri,
 Gyorgy; Orfi, Laszlo; Szekely, Istvan; Choidas, Axel;
 Bacher, Gerald
 PATENT ASSIGNEE(S): Axxima Pharmaceuticals A.-G., Germany
 SOURCE: PCT Int. Appl., 127 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|--|----------|-----------------|------------|
| WO 2001056553 | A2 | 20010809 | WO 2001-EP1126 | 20010202 |
| WO 2001056553 | A3 | 20020328 | | |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |
| EP 1255541 | A2 | 20021113 | EP 2001-911580 | 20010202 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | |
| PRIORITY APPLN. INFO.: | | | EP 2000-102050 | A 20000202 |
| | | | US 2000-179795P | P 20000202 |
| | | | WO 2001-EP1126 | W 20010202 |
| OTHER SOURCE(S): | MARPAT 135:162484 | | | |
| AB | The present invention provides arom. guanylhydrazone compds. and their use as pharmaceutically active agents, esp. for prophylaxis and treatment of virally caused diseases and infections, including opportunistic infections. The guanylhydrazone compds. are also useful as inhibitors of deoxyhypusine synthase and as inhibitors for nuclear export in infectious diseases and may be used to regulate bacterially induced TNF-.alpha. prodn. Furthermore, the arom. guanylhydrazones exhibit antibacterial activity against Gram-pos. and Gram-neg. bacteria and can be regarded as a novel class of antibiotics. In addn., methods for prophylaxis and treatment of virally or bacterially induced infections and diseases are disclosed, together with pharmaceutical compns. useful within the methods contg. at least one arom. guanylhydrazone of the invention as active ingredient. | | | |

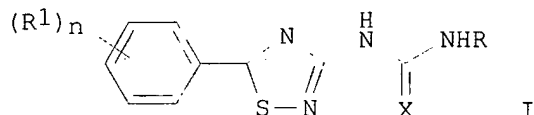
L17 ANSWER 18 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
 ACCESSION NUMBER: 2001:499858 HCAPLUS
 DOCUMENT NUMBER: 135:87152
 TITLE: A method and pharmaceutical composition using
 (5-aryl-1,2,4-thiadiazol)-3-yl thiourea derivatives or
 (5-aryl-1,2,4-thiadiazol)-3-yl urea derivatives as

antiviral, antineoplastic, and antifungal agents
 INVENTOR(S): Camden, James Berger
 PATENT ASSIGNEE(S): The Procter & Gamble Company, USA
 SOURCE: U.S., 13 pp., Cont.-in-part of U.S. Ser. No. 281,896,
 abandoned.
 CODEN: USXXAM
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|------|----------|-----------------|----------|
| US 6258831 | B1 | 20010710 | US 2000-535172 | 20000327 |
| US 2001031773 | A1 | 20011018 | US 2001-812094 | 20010319 |
| US 6410575 | B1 | 20020625 | | |

PRIORITY APPLN. INFO.: US 1999-281896 B2 19990331
 US 2000-535172 A3 20000327

OTHER SOURCE(S): MARPAT 135:87152
 GI



AB Methods are disclosed for the treatment of viral infections, particularly hepatitis C, herpes simplex, Kaposi's sarcoma and HIV, comprising administering a (5-aryl-1,2,4-thiadiazol)-3-yl thiourea deriv. or (5-aryl-1,2,4-thiadiazol)-3-yl urea deriv. (I) [X=O,S; R=H,C1-3 alkyl; , n=0-4; R1=H, C1-7 alkyl, Cl, Br or F, oxychloro, O(CH2)yCH3 (Y=1-6)] or a pharmaceutically acceptable acid addn. salt or prodrug thereof. The preferred compd. is (5-phenyl-1,2,4-thiadiazol-3-yl) thiourea. A method for the treatment of fungal infection is also presented.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 19 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
 ACCESSION NUMBER: 2000:911455 HCAPLUS
 DOCUMENT NUMBER: 134:66119
 TITLE: Method of screening for strains of human immunodeficiency virus 2 resistant to proteinase inhibitors in a biological sample taken from a patient
 INVENTOR(S): Telles, Jean-Noel; Brun-Vezinet, Francoise; Descamps, Diane
 PATENT ASSIGNEE(S): Bio Merieux, Fr.; Assistance Publique - Hopitaux de Paris
 SOURCE: PCT Int. Appl., 19 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: French
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|------|-----------------|------|
| | | | | |

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WO 2000078990      A2      20001228      WO 2000-FR1728      20000621
WO 2000078990      A3      20010301
W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
    CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
    HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
    LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
    SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
    YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW:  GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
    DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
    CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
FR 2798385          A1      20010316      FR 1999-7855      19990621
FR 2798385          B1      20030905
AU 2000062875       A5      20010109      AU 2000-62875      20000621
EP 1185713          A2      20020313      EP 2000-949555      20000621
R:   AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
    IE, SI, LT, LV, FI, RO
PRIORITY APPLN. INFO.:      FR 1999-7855      A      19990621
                                WO 2000-FR1728      W      20000621
AB  A method of screening sample contg. HIV-2 for possible
    resistance to antiviral proteinase inhibitors by screening for alleles of
    the proteinase gene is described. Hybridization probes for the detection
    of mutations in the gene known to lead to drug resistance are described.
    If said mutation is obsd., the existence of a resistance to said anti-
protease agent is assumed in the patient.

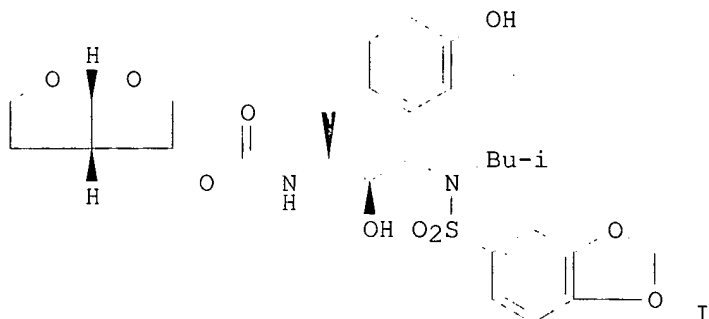
L17  ANSWER 20 OF 51  HCAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:      2000:900607  HCAPLUS
DOCUMENT NUMBER:      134:56676
TITLE:      Preparation of arylsulfonamides as inhibitors
            of aspartyl protease
INVENTOR(S):      Hale, Michael Robin; Tung, Roger; Price, Stephen;
            Wilkes, Robin David; Schairer, Wayne Carl; Jarvis,
            Ashley Nicholas; Spaltenstein, Andrew; Furfine, Eric
            Steven; Samano, Vicente; Kaldor, Istvan; Miller, John
            Franklin; Brieger, Michael Stephen
PATENT ASSIGNEE(S):      Vertex Pharmaceuticals Inc., USA; et al.
SOURCE:      PCT Int. Appl., 396 pp.
            CODEN: PIXXD2
DOCUMENT TYPE:      Patent
LANGUAGE:      English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

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| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|--|----------|-----------------|----------|
| WO 2000076961 | A1 | 20001221 | WO 2000-US15781 | 20000608 |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |
| BR 2000011745 | A | 20020319 | BR 2000-11745 | 20000608 |
| EP 1194404 | A1 | 20020410 | EP 2000-941279 | 20000608 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, | | | |

Stucker 09/980,777

IE, SI, LT, LV, FI, RO
JP 2003502309 T2 20030121 JP 2001-503821 20000608
NO 2001006034 A 20020118 NO 2001-6034 20011210
PRIORITY APPLN. INFO.: US 1999-139070P P 19990611
US 2000-190211P P 20000317
WO 2000-US15781 W 20000608
OTHER SOURCE(S): MARPAT 134:56676
GI



AB The title arylsulfonamides, namely (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl 3-arylsulfonylamino-1-(4-hydroxyphenyl)-2-hydroxypropylcarbamate derivs. (e.g. I) are prepd. These compds. are particularly well suited for inhibiting HIV-1 and **HIV-2 protease** activity and consequently, may be advantageously used as anti-viral agents against the HIV-1 and HIV-2 viruses. They are useful for treating with a patient diagnosed with AIDS, AIDS related complex (ARC), progressive generalized lymphadenopathy (PGL), Kaposi's sarcoma, thrombocytopenic purpura, or AIDS-related neurol. conditions such as AIDS dementia complex, multiple sclerosis or tropical paraperesis, etc. Thus, (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl 3-[N-(1,3-benzodioxol-5-ylsulfonyl)-N-isobutylamino]-1-(4-hydroxyphenyl)-2-hydroxypropylcarbamate underwent Mitsunobu reaction with phenethyl alc. using Ph3P and di-tert-Bu azodicarbonate in CH₂Cl₂ at room temp. for 1.5 h to give 72% I. I showed IC₅₀ of <0.001, <0.001, and 0.01-0.001 .mu.M against drug-resistant HIV strains, i.e. wild type, mutant HIV-1 EP13, and mutant D545701-14 HIV strains, resp., in MT-4 cells.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 21 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:706974 HCAPLUS
DOCUMENT NUMBER: 133:286468
TITLE: Viral treatment using 2-thienyl-imidazolo[4,5]pyridine derivatives
INVENTOR(S): Camden, James Berger; Gardner, Joseph Herman; Stanton, David Thomas
PATENT ASSIGNEE(S): The Procter & Gamble Company, USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

Stucker 09/980,777

FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|--|----------|-----------------|------------|
| WO 2000057870 | A2 | 20001005 | WO 2000-US9419 | 20000329 |
| WO 2000057870 | A3 | 20010308 | | |
| W: | AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |
| EP 1165077 | A2 | 20020102 | EP 2000-919868 | 20000329 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | |
| JP 2002540151 | T2 | 20021126 | JP 2000-607621 | 20000329 |
| AU 763431 | B2 | 20030724 | AU 2000-40489 | 20000329 |
| PRIORITY APPLN. INFO.: | | | US 1999-281892 | A 19990331 |
| | | | WO 2000-US8419 | W 20000329 |

OTHER SOURCE(S): MARPAT 133:286468

AB A pharmaceutical compn. that inhibits or slows the growth of viruses is disclosed. This same compn. can be used to treat viral infections, particularly HIV and hepatitis as well as fungal infections with *Cryptococcus neoformans* or *Curvularia lunata*. The compn. comprises .apprx. 10-6000 mg of a 2-thienyl-imidazolo[4,5]pyridine deriv., and prodrugs and pharmaceutically acceptable salts thereof. The preferred antiviral compd. is 2-(2-thienyl)-imidazolo[4,5-b]pyridine (I) and its hydrochloride salt. Mixts. of the 2-thienyl-imidazolo[4,5]pyridines can also be used to treat viral infections. For example, in an in vitro virus prodn. test of hepatitis B, HEPG2 2.2215 the following results were obtained for I (0.64-200 .mu.g/mL): IC50 = 1.6 .mu.g/mL, TC50 = 16.3 .mu.g/mL, and therapeutic index TI = 10.1; in a replicate expt., the values were IC50 = 10.71 .mu.g/mL, TC50 = 16.8 .mu.g/mL and TI = 23.4. For comparison, 3TC (0.0032-0.32 .mu.g/mL) was tested with the following results: IC50 = 0.089 .mu.g/mL, TC50 > 1 .mu.g/mL, and TI = 14.6; in a replicate expt., the values were the IC50 = 0.021 .mu.g/mL, TC50 1 .mu.g/mL, and TI = > 47.6. Thus, 2-(2-thienyl)-imidazolo[4,5-b]pyridine can be used to treat hepatitis B.

L17 ANSWER 22 OF 51 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:41229 BIOSIS

DOCUMENT NUMBER: PREV200100041229

TITLE: Proteasome inhibition interferes with Gag polyprotein processing, release, and maturation of HIV-1 and HIV-2.
AUTHOR(S): Schubert, Ulrich (1); Ott, David E.; Chertova, Elena N.; Welker, Reinhold; Tessmer, Uwe; Princiotta, Michael F.; Bennink, Jack R. (1); Kraeusslich, Hans-Georg; Yewdell, Jonathan W. (1)

CORPORATE SOURCE: (1) Laboratory of Viral Diseases, National Institutes of Health, 4 Center Drive, Room 209 Building 4, MSC 0440, Bethesda, MD, 20892-0440: uschubert@nih.gov, jrbennink@nih.gov, jyewdell@nih.gov USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (November 21, 2000) Vol. 97, No. 24, pp. 13057-13062. print.

ISSN: 0027-8424.

DOCUMENT TYPE: Article
 LANGUAGE: English
 SUMMARY LANGUAGE: English

AB Retrovirus assembly and maturation involve folding and transport of viral proteins to the virus assembly site followed by subsequent proteolytic cleavage of the Gag polyprotein within the nascent virion. We report that inhibiting proteasomes severely decreases the budding, maturation, and infectivity of HIV. Although processing of the Env glycoproteins is not changed, proteasome inhibitors inhibit processing of Gag polyprotein by the viral **protease** without affecting the activity of the HIV-1 viral **protease** itself, as demonstrated by in vitro processing of HIV-1 Gag polyprotein Pr55. Furthermore, this effect occurs independently of the virus release function of the HIV-1 accessory protein Vpu and is not limited to HIV-1, as proteasome inhibitors also reduce virus release and Gag processing of HIV-2. Electron microscopy analysis revealed ultra-structural changes in budding virions similar to mutants in the late assembly domain of p6gag, a C-terminal domain of Pr55 required for efficient virus maturation and release. Proteasome inhibition reduced the level of free ubiquitin in HIV-1 infected cells and prevented monoubiquitination of p6gag. Consistent with this, viruses with mutations in PR or p6gag were ~~resistant~~ to detrimental effects mediated by proteasome inhibitors. These results indicate the requirement for an active proteasome/ubiquitin system in release and maturation of infectious HIV particles and provide a potential pharmaceutical strategy for interfering with retrovirus replication.

L17 ANSWER 23 OF 51 MEDLINE on STN DUPLICATE 3

ACCESSION NUMBER: 2000483572 MEDLINE
 DOCUMENT NUMBER: 20457136 PubMed ID: 11000004
 TITLE: Synthesis, stability, antiviral activity, and **protease**-bound structures of substrate-mimicking constrained macrocyclic **inhibitors** of HIV-1 **protease**.

AUTHOR: Tyndall J D; Reid R C; Tyssen D P; Jardine D K; Todd B; Passmore M; March D R; Pattenden L K; Bergman D A; Alewood D; Hu S H; Alewood P F; Birch C J; Martin J L; Fairlie D P
 CORPORATE SOURCE: Centre for Drug Design and Development, The University of Queensland, Brisbane, Queensland 4072, Australia.

SOURCE: JOURNAL OF MEDICINAL CHEMISTRY, (2000 Sep 21) 43 (19) 3495-504.
 Journal code: 9716531. ISSN: 0022-2623.

PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 200010
 ENTRY DATE: Entered STN: 20001019
 Last Updated on STN: 20001019
 Entered Medline: 20001012

AB Three new peptidomimetics (1-3) have been developed with highly stable and conformationally constrained macrocyclic components that replace tripeptide segments of **protease** substrates. Each compound **inhibits** both HIV-1 **protease** and viral replication (HIV-1, HIV-2) at nanomolar concentrations without cytotoxicity to uninfected cells below 10 microM. Their activities against HIV-1 **protease** (K(i) 1.7 nM (1), 0.6 nM (2), 0.3 nM (3)) are 1-2 orders of magnitude greater than their antiviral potencies against HIV-1-infected primary peripheral blood mononuclear cells (IC(50) 45 nM

(1), 56 nM (2), 95 nM (3)) or HIV-1-infected MT2 cells (IC₅₀ 90 nM (1), 60 nM (2)), suggesting suboptimal cellular uptake. However their antiviral potencies are similar to those of indinavir and amprenavir under identical conditions. There were significant differences in their capacities to inhibit the replication of HIV-1 and HIV-2 in infected MT2 cells, 1 being ineffective against HIV-2 while 2 was equally effective against both virus types. Evidence is presented that 1 and 2 inhibit cleavage of the HIV-1 structural protein precursor Pr55(gag) to p24 in virions derived from chronically infected cells, consistent with **inhibition** of the viral **protease** in cells. Crystal structures refined to 1.75 Å (1) and 1.85 Å (2) for two of the macrocyclic inhibitors bound to HIV-1 **protease** establish structural mimicry of the tripeptides that the cycles were designed to imitate. Structural comparisons between **protease**-bound macrocyclic **inhibitors**, VX478 (amprenavir), and L-735,524 (indinavir) show that their common acyclic components share the same space in the active site of the enzyme and make identical interactions with enzyme residues. This substrate-mimicking minimalist approach to drug design could have benefits in the context of viral **resistance**, since mutations which induce inhibitor **resistance** may also be those which prevent substrate processing.

L17 ANSWER 24 OF 51 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4

ACCESSION NUMBER: 2000:447407 BIOSIS
DOCUMENT NUMBER: PREV200000447407
TITLE: Nonnucleoside human cytomegalovirus inhibitors: Synthesis and antiviral evaluation of (chlorophenylmethyl)benzothiadiazine dioxide derivatives.
AUTHOR(S): Martinez, Ana (1); Gil, Carmen; Perez, Concepcion; Castro, Ana; Prieto, Columbiana; Otero, Joaquin; Andrei, Graciela; Snoeck, Robert; Balzarini, Jan; De Clercq, Erik
CORPORATE SOURCE: (1) Instituto de Quimica Medica, CSIC, Juan de la Cierva 3, 28006, Madrid Spain
SOURCE: Journal of Medicinal Chemistry, (August 24, 2000) Vol. 43, No. 17, pp. 3267-3273. print.
ISSN: 0022-2623.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A second generation of benzothiadiazine dioxide (BTD) derivatives was synthesized employing benzylation reactions mainly. The chlorophenylmethyl BTD derivatives showed activity against human cytomegalovirus (HCMV) with IC₅₀ values ranging from 3 to 10 µM. Their 50% cytotoxic concentrations were often >200 µM to lung fibroblast HEL cell proliferation and between 20 and 35 µM for lymphocyte CME cell growth. When cytotoxicity for cell morphology was considered, the minimum cytotoxic concentration for the different BTD derivatives varied between 5 and 200 µM. Some of the anti-HCMV compounds also showed activity against HIV-1 and HIV-2. The chlorophenylmethyl derivative 21 was active against a variety of HCMV clinical isolates from patients with different clinical manifestations and fully maintained its activity against a ganciclovir-**resistant** HCMV strain. The dibenzyl BTD derivatives did not **inhibit** HCMV **protease**, and preliminary pharmacological experiments revealed that their anti-HCMV action stems from interference with an early stage of the viral replicative cycle.

L17 ANSWER 25 OF 51 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2000211658 MEDLINE

DOCUMENT NUMBER: 20211656 PubMed ID: 10747109
 TITLE: Emergence of drug **resistance** mutations in human immunodeficiency virus type 2-infected subjects undergoing antiretroviral therapy.
 AUTHOR: Rodes B; Holguin A; Soriano V; Dourana M; Mansinho K; Antunes F; Gonzalez-Lahoz J
 CORPORATE SOURCE: Service of Infectious Diseases, Hospital Carlos III, Instituto de Salud Carlos III, Madrid, Spain.
 SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (2000 Apr) 38 (4) 1370-4. Journal code: 7505564. ISSN: 0095-1137.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 OTHER SOURCE: GENBANK-AF139042; GENBANK-AF139043; GENBANK-AF139044; GENBANK-AF139045; GENBANK-AF139046; GENBANK-AF139047; GENBANK-AF139048; GENBANK-AF139049; GENBANK-AF139050; GENBANK-AF139051; GENBANK-AF139052; GENBANK-AF139053; GENBANK-AF139054
 ENTRY MONTH: 200005
 ENTRY DATE: Entered STN: 20000525
 Last Updated on STN: 20000525
 Entered Medline: 20000517

AB The reverse transcriptase (RT) and **protease** genes from 12 human immunodeficiency virus type 2 (HIV-2)-infected individuals who had been exposed to antiretroviral drugs for longer than 6 months were examined for the presence of mutations which could be involved in drug **resistance**. Four individuals carried virus genotypes with amino acid substitutions potentially associated with **resistance** to nucleoside analogues: two at codon 70 (K-->R) and two at codon 184 (M-->V). Moreover, the latter two patients harbored a codon Q151M mutation which is associated to multidrug **resistance** in HIV-1, and one of these subjects carried some of the typically linked mutations at codons 65 and 69. With regard to the **protease inhibitors**, substitutions associated with **resistance** to **protease inhibitors** at codon 46 were observed in all individuals. Moreover, minor **resistance** mutations, as well as new ones of unknown meaning, were often seen in the **protease** gene. In conclusion, amino acid changes in the HIV-2 RT and **protease** genes which could be associated with drug **resistance** seem to occur at positions identical to those for HIV-1.

L17 ANSWER 26 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2000:776360 HCAPLUS
 DOCUMENT NUMBER: 134:95156
 TITLE: Polyanionic (i.e., polysulfonate) dendrimers can inhibit the replication of human immunodeficiency virus by interfering with both virus adsorption and later steps (reverse transcriptase/integrase) in the virus replicative cycle
 AUTHOR(S): Wilveroux, Myriam; Fikkert, Valery; Pluymers, Wim; Matthews, Barry; Mardel, Karen; Schols, Dominique; Raff, John; Debyser, Zeger; De Clercq, Erik; Holan, George; Pannecouque, Christophe
 CORPORATE SOURCE: Rega Institute for Medical Research, Katholieke Universiteit Leuven, Louvain, Belg.
 SOURCE: Molecular Pharmacology (2000), 58(5), 1100-1108
 CODEN: MOPMA3; ISSN: 0026-895X

PUBLISHER: American Society for Pharmacology and Experimental
Therapeutics
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Polyanionic dendrimers were synthesized and evaluated for their antiviral effects. Phenylldicarboxylic acid (BRI6195) and naphthylldisulfonic acid (BRI2923) dendrimers were found to inhibit the replication of human immunodeficiency virus type 1 (HIV-1; strain IIIB) in MT-4 cells at a EC50 of 0.1 and 0.3 .mu.g/mL, resp. The dendrimers were not toxic to MT-4 cells up to the highest concns. tested (250 .mu.g/mL). These compds. were also effective against various other HIV-1 strains, including clin. isolates, HIV-2 strains, simian immunodeficiency virus (SIV, strain MAC251), and HIV-1 strains that were **resistant** to reverse transcriptase inhibitors. HIV strains contg. mutations in the envelope glycoprotein gp120 (engendering **resistance** to known adsorption inhibitors) displayed reduced sensitivity to the dendrimers. The compds. inhibited the binding of wild-type virus and recombinant virus (contg. wild-type gp120) to MT-4 cells at concns. comparable to those that inhibited the replication of HIV-1(IIIB) in these cells. Cellular uptake studies indicated that BRI2923, but not BRI6195, permeates into MT-4 and CEM cells. Accordingly, the naphthylldisulfonic acid dendrimer (BRI2923) proved able to inhibit later steps of the replication cycle of HIV, i.e., reverse transcriptase and integrase. NL4.3 strains **resistant** to BRI2923 were selected after passage of the virus in the presence of increasing concns. of BRI2923. The virus mutants showed 15-fold reduced sensitivity to BRI2923 and cross-**resistance** to known adsorption inhibitors. However, these virus mutants were not cross-**resistant** to reverse transcriptase **inhibitors** or **protease inhibitors**. We identified several mutations in the envelope glycoprotein gp120 gene (i.e., V2, V3, and C3, V4, and C4 regions) of the BRI2923-**resistant** NL4.3 strains that were not present in the wild-type NL4.3 strain, whereas no mutations were found in the reverse transcriptase or integrase genes.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 27 OF 51 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 6

ACCESSION NUMBER: 2000237078 EMBASE
TITLE: Amprenavir: A new human immunodeficiency virus type 1 **protease inhibitor**.
AUTHOR: Fung H.B.; Kirschenbaum H.L.; Hameed R.
CORPORATE SOURCE: Dr. H.B. Fung, Pharmacy Service, Bronx VA Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, United States
SOURCE: Clinical Therapeutics, (2000) 22/5 (549-572).
Refs: 68
ISSN: 0149-2918 CODEN: CLTHDG
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 030 Pharmacology
037 Drug Literature Index
038 Adverse Reactions Titles
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Objective: This paper reviews the pharmacologic properties and clinical usefulness of amprenavir, a new human immunodeficiency virus type 1 (HIV-1) **protease inhibitor**. Background: Amprenavir, the most recent HIV-1 **protease inhibitor** to receive marketing approval from the US Food and Drug Administration, is a potent

competitive inhibitor of HIV-1 protease and a relatively weak inhibitor of HIV-2 protease. Inhibition of the HIV-I protease enzyme results in immature and noninfectious viral particles. Amprenavir is rapidly absorbed following oral administration. The time to peak concentration (T(max)) in adults is between 1 and 2 hours, the area under the plasma concentration versus time curve is roughly proportional to the dose, the half-life is .apprx.8 hours, and the volume of distribution is .apprx.430 L. The T(max) in children 4 to 12 years of age is between 1.1 and 1.4 hours. The bioavailability of the solution is 86% relative to the capsule formulation. It is metabolized by the cytochrome P-450 isozyme CYP3A4 and to a lesser extent by CYP2D6 and CYP2C9. Methods: We searched MEDLINE.RTM. (1966 to January 2000), AIDSLINE.RTM. (1980 to January 2000), International Pharmaceutical Abstracts (1970 to January 2000), PharmaProjects (January 2000 version), and web sites of major HIV/acquired immunodeficiency syndrome conferences for appropriate published references (1996 to February 2000). Results: Data reported to date indicate that amprenavir is efficacious in the treatment of HIV disease in patients with primary HIV infection, antiretroviral-naive patients, **protease inhibitor**-naive patients, **protease inhibitor**-experienced patients, and pediatric patients. Adverse effects were usually of early onset (range, 2 to 21 days) and transient (range, 3 to 46 days), although the incidence of metabolic abnormalities such as lipodystrophy, hyperlipidemia, and diabetes mellitus has not yet been defined. Amprenavir should be avoided in patients with a known sulfonamide allergy. Concomitant use of other medications that are CYP3A4 inducers or inhibitors should be done cautiously and only if the potential benefit clearly outweighs potential risk. The dose should be reduced in patients with significant hepatic impairment (Child-Pugh score, .gtoreq.5). Amprenavir probably should not be administered with rifabutin, rifampin, astemizole, midazolam, triazolam, bepridil, dihydroergotamine, ergotamine, or cisapride. The recommended adult dose is 1200 mg twice daily. For patients between 4 and 12 years of age or between 13 and 16 years of age who weigh <50 kg, the recommended dosage of the capsule form is 20 mg/kg (22.5 mg/kg for oral solution) twice daily or 15 mg/kg (17 mg/kg for oral solution) 3 times a day to a maximum dose of 2400 mg (2800 mg for oral solution). Patients should not take vitamin E supplements because amprenavir is formulated with a large amount of vitamin E (109 IU/capsule and 46 IU/mL oral solution) to improve oral absorption. Amprenavir may be administered with or without food, but a high-fat meal (>67 g fat) should be avoided. Conclusions: Published clinical data are limited, but amprenavir appears to be efficacious and generally well tolerated in patients with HIV infection. Pharmacoeconomic data are not yet available. The introduction of amprenavir appears to be important, since it provides an additional treatment option as a component of both initial and salvage combination therapies for patients with HIV.

L17 ANSWER 28 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN
 ACCESSION NUMBER: 1999:819523 HCAPLUS
 DOCUMENT NUMBER: 132:59135
 TITLE: Fitness assay and associated methods, and applications to drug **resistance** and HIV **protease inhibitors** and other drugs with reduced **resistance**
 INVENTOR(S): Erickson, John W.; Gulnik, Sergei V.
 PATENT ASSIGNEE(S): United States of America, Represented by the Secretary, Department of Health and Human Services, USA
 SOURCE: PCT Int. Appl., 119 pp.

CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|------------|
| WO 9967417 | A2 | 19991229 | WO 1999-US14119 | 19990623 |
| WO 9967417 | A3 | 20000928 | | |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2336160 | AA | 19991229 | CA 1999-2336160 | 19990623 |
| AU 9948280 | A1 | 20000110 | AU 1999-48280 | 19990623 |
| EP 1088098 | A2 | 20010404 | EP 1999-931861 | 19990623 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 2002518063 | T2 | 20020625 | JP 2000-556057 | 19990623 |
| PRIORITY APPLN. INFO.: | | | US 1998-90393P | P 19980623 |
| | | | WO 1999-US14119 | W 19990623 |

OTHER SOURCE(S): MARPAT 132:59135

GI For diagram(s), see printed CA Issue.

AB The invention provides an assay for detg. the biochem. fitness of a biochem. species in a mutant replicating biol. entity relative to its predecessor. The invention further provides a continuous fluorogenic assay for measuring the anti-HIV **protease** activity of **protease inhibitor**. The invention also provides a method of administering a therapeutic compd. that reduces the chances of the emergence of drug **resistance** in therapy. The invention also provides a compd. AXQN(R2)CH[(CH2)mR3]CH(R4)CH2N(R5)(WR6) [A = Q1, Q2, Q3, Q4; R1, R2, R3, R5, R6 = H, (substituted and/or heteroatom-bearing) alkyl, alkenyl, alkynyl, or cyclic group; Y, Z = CH2, O, S, SO, SO2, amino, amides, carbamates, ureas, or thiocarbonyl derivs. thereof, optionally substituted with an alkyl, alkenyl, or alkynyl group; n = 1-5; X = bond, (substituted) methylene or ethylene, amino, O, S; Q = C(O), C(S), SO2; m = 0-6; R4 = OH, =O (keto), NH2, alkylamino, including esters, amides, and salts thereof; W = C(O), C(S), S(O), SO2; Optionally, R5 and R6, together with the NW bond comprise a macrocyclic ring], or a pharmaceutically acceptable salt, a prodrug, a compn., or an ester thereof.

L17 ANSWER 29 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 7

ACCESSION NUMBER: 1999:477765 HCAPLUS

DOCUMENT NUMBER: 131:237541

TITLE: JE-2147: a dipeptide **protease inhibitor** (PI) that potently inhibits multi-PI-**resistant** HIV-1

AUTHOR(S): Yoshimura, Kazuhisa; Kato, Ryohei; Yusa, Keisuke; Kavlick, Mark F.; Maroun, Victor; Nguyen, Aline; Mimoto, Tsutomu; Ueno, Takamasa; Shintani, Makoto; Falloon, Judith; Masur, Henry; Hayashi, Hideya; Erickson, John; Mitsuya, Hiroaki

CORPORATE SOURCE: Experimental Retrovirology Section, Medicine Branch,

Division of Clinical Sciences, National Cancer
Institute, National Institutes of Health, Bethesda,
MD, 20892, USA

SOURCE: Proceedings of the National Academy of Sciences of the
United States of America (1999), 96(15), 8675-8680
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors designed, synthesized, and identified JE-2147, an
allophenylnorstatine-contg. dipeptide HIV **protease**
inhibitor (PI), which is potent against a wide spectrum of HIV-1,
HIV-2, simian immunodeficiency virus, and various clin. HIV-1 strains in
vitro. Drug-**resistant** clin. HIV-1 strains, isolated from seven
patients who had failed 9-11 different anti-HIV therapeutics after 32-83
mo, had a variety of drug-**resistance**-related amino acid
substitutions and were highly and invariably **resistant** to all of
the currently available anti-HIV agents. JE-2147 was, however, extremely
potent against all such drug-**resistant** strains, with IC50 values
ranging from 13-41 nM (2-fold changes in IC50 compared with that of
wild-type HIV-1). The emergence of JE-2147-**resistant** HIV-1
variants in vitro was substantially delayed compared with that of HIV-1
resistant to another allophenylnorstatine-contg. compd., KNI-272,
and other related PIs. Structural anal. revealed that the presence of a
flexible P2' moiety is important for the potency of JE-2147 toward
wild-type and mutant viruses. These data suggest that the use of flexible
components may open a new avenue for designing PIs that resist the
emergence of PI-**resistant** HIV-1. Further development of JE-2147
for treating patients harboring multi-PI-**resistant** HIV-1 is
warranted.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 30 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1999:738016 HCAPLUS

DOCUMENT NUMBER: 132:44542

TITLE: Inhibition of adipocyte differentiation by HIV
protease inhibitors

AUTHOR(S): Zhang, Bei; Macnaul, Karen; Szalkowski, Deborah; Li,
Zhihua; Berger, Joel; Moller, David E.

CORPORATE SOURCE: Department of Metabolic Disorders, Merck Research
Laboratories, Rahway, NJ, 07065, USA

SOURCE: Journal of Clinical Endocrinology and Metabolism
(1999), 84(11), 4274-4277

CODEN: JCEMAZ; ISSN: 0021-972X

PUBLISHER: Endocrine Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Patients with AIDS who are receiving therapy with HIV **protease**
inhibitors have been widely reported to be afflicted with a
syndrome characterized by lipodystrophy (fat redistribution favoring the
accumulation of abdominal and cervical adipose tissue), hyperlipidemia,
and insulin **resistance**. HIV **protease**
inhibitors have been suggested to have a direct role in modulating
adipocyte differentiation. To address this hypothesis, several HIV
protease inhibitors were studied for their ability to
either augment or inhibit the differentiation of murine 3T3-L1
preadipocytes. Dose-responsive inhibition of adipogenesis by several
protease inhibitors was noted as measured by reduced

triglyceride accumulation and attenuated induction of three differentiation marker genes - aP2, lipoprotein lipase, and Adipo Q. Potential mechanisms for altered adipocyte function, including direct binding to PPAR.gamma. or inhibition of PPAR.gamma.-mediated gene transcription were effectively excluded.

REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 31 OF 51 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

ACCESSION NUMBER: 2000:3241 SCISEARCH

THE GENUINE ARTICLE: 266RP

TITLE: Inhibition of the HIV-1 and HIV-2
proteases by a monoclonal antibody

AUTHOR: Lescar J; Brynda J; Rezacova P; Stouracova R; Riottot M M;
Chitarra V; Fabry M; Horejsi M; Sedlacek J; Bentley G A
(Reprint)

CORPORATE SOURCE: INST PASTEUR, DEPT IMMUNOL, UNITE IMMUNOL STRUCT, CNRS,
URA 1961, F-75724 PARIS 15, FRANCE (Reprint); INST
PASTEUR, DEPT IMMUNOL, UNITE IMMUNOL STRUCT, CNRS, URA
1961, F-75724 PARIS 15, FRANCE; ACAD SCI CZECH REPUBL,
INST MOL GENET, DEPT GENE MANIPULAT, CR-16637 PRAGUE 6,
CZECH REPUBLIC; EUROPEAN SYNCHROTRON RADIAT FACIL, F-38043
GRENOBLE, FRANCE

COUNTRY OF AUTHOR: FRANCE; CZECH REPUBLIC

SOURCE: PROTEIN SCIENCE, (DEC 1999) Vol. 8, No. 12, pp. 2686-2696.
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW
YORK, NY 10011-4211.
ISSN: 0961-8368.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 57

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The monoclonal antibody 1696, directed against the HIV-1

protease, displays strong **inhibitory** effects toward the catalytic activity of the enzyme of both the HIV-1 and HIV-2 isolates. This antibody cross-reacts with peptides that include the N-terminus of the enzyme, a region that is well conserved in sequence among different viral strains and which, furthermore, is crucial for homodimerization to the active enzymatic form. This observation, as well as antigen-binding studies in the presence of an active site inhibitor, suggest that 1696 **inhibits** the HIV **protease** by destabilizing its active homodimeric form. To characterize further how the antibody 1696 inhibits the HIV-1 and **HIV-2 proteases**, we have solved the crystal structure of its Fab fragment by molecular replacement and refined it at 3.0 Angstrom, resolution. The antigen binding site has a deep cavity at its center, which is lined mainly by acidic and hydrophobic residues, and is large enough to accommodate several antigen residues. The structure of the Fab 1696 could form a starting basis for the design of alternative HIV **protease-inhibiting** molecules of broad specificity.

L17 ANSWER 32 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 8

ACCESSION NUMBER: 1999:633775 HCAPLUS

DOCUMENT NUMBER: 131:280910

TITLE: Antiretroviral agent nevirapine. Its pharmacological
action and potential for clinical use

AUTHOR(S): Takeuchi, Shougo; Osugi, Takeshi

CORPORATE SOURCE: Dep. Pharmacol., Kawanishi Pharma Res. Inst., Nippon

Boehringer Ingelheim Co., Ltd., 3-10-1, Yato,
Kawanishi, 666-0193, Japan
SOURCE: Nippon Yakurigaku Zasshi (1999), 114(4), 205-211
CODEN: NYKZAU; ISSN: 0015-5691
PUBLISHER: Nippon Yakuri Gakkai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese

AB A review with 26 refs. Nevirapine (NVP) is a potent noncompetitive inhibitor of the reverse transcriptase enzyme, which is necessary for HIV replication. NVP selectively inhibits HIV-1 but not HIV-2 and any of the human DNA polymerases. NVP is active against ZDV-resistant HIV-1 and synergistic with non-nucleoside reverse transcriptase inhibitors. NVP has a favorable pharmacokinetic profile, becomes widely distributed throughout body tissues including the central nervous system, and is active in the adult at an oral dose of 200 mg administered twice daily after a 2 wk lead-in dose of 200 mg/day due to its long elimination half life. Although the currently used **protease inhibitors** (PIs) may undergo more rapid rates of metab. because NVP induces CYP3A, No dosage adjustments are required when NVP is taken in combination with PIs so far. When administered in triple combinations with antiretroviral agents. the antiviral effect of NVP has been profound and sustained in HIV-infected patients, particularly in naive patients to antiretroviral therapy. **Resistance** to NVP is rapid when given as monotherapy, but this is altered and made less clin. relevant when NVP is administered as a triple combination. NVP has a safety profile that does not overlap with other antiretroviral therapies, the most common treatment-limiting reaction being rash. It seems that NVP would be a very useful option in combination with antiretroviral agents.

L17 ANSWER 33 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 9

ACCESSION NUMBER: 1999:168106 HCAPLUS
DOCUMENT NUMBER: 130:346905
TITLE: PMTI, a broadly active unusual single-stranded polyribonucleotide, inhibits human immunodeficiency virus replication by multiple mechanisms
AUTHOR(S): Buckheit, Robert W., Jr.; Lackman-Smith, Carol; Snow, Melinda J.; Halliday, Susan M.; White, E. Lucile; Ross, Larry J.; Agrawal, Vijai K.; Broom, Arthur D.
CORPORATE SOURCE: Microbiology Research Department, Southern Research Institute, Frederick, MD, 21701, USA
SOURCE: Antiviral Chemistry & Chemotherapy (1999), 10(1), 23-32
CODEN: ACCHEH; ISSN: 0956-3202
PUBLISHER: International Medical Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Poly(1-methyl-6-thioinosinic acid), or PMTI, is a single-stranded polyribonucleotide and is the first homo polyribonucleotide devoid of Watson-Crick hydrogen bonding sites to show potent human immunodeficiency virus (HIV) inhibition. PMTI was active when evaluated against a variety of low passage clin. HIV isolates in fresh human peripheral blood cells, including T cell-tropic and monocyte-macrophage-tropic viruses, syncytium-inducing and non-syncytium-inducing viruses and viruses representative of the various HIV-1 clades (A through F). The compd. was active against HIV-2, all nucleoside and non-nucleoside reverse transcriptase (RT) inhibitor drug-resistant virus isolates tested and interacted with AZT or ddI to synergistically inhibit HIV infection. In biochem. inhibition assays, PMTI was detd. to be a potent inhibitor of HIV-1 and HIV-2 RT, including RTs with mutations that

engender **resistance** to nucleoside and non-nucleoside RT inhibitors. PMTI inhibited both the polymerase and RNase H activities of HIV RT. PMTI did not **inhibit** HIV-1 **protease** or integrase. Cell-based mechanism of action assays indicated that PMTI also interfered with early events in the entry of HIV into target cells. Furthermore, PMTI inhibited the fusion of gp120-expressing and CD4-expressing cells, but at concns. approx. 1 log10 greater than those that inhibited virus entry. These results suggest that the homo polyribonucleotide PMTI blocks HIV replication in human cells at its earliest stages by multiple mechanisms, inhibition of virus entry and inhibition of RT.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 34 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1998:65902 HCAPLUS

DOCUMENT NUMBER: 128:123799

TITLE: Antiviral pharmaceutical compositions containing saturated 1,2-dithiaheterocyclic compounds, and uses thereof

INVENTOR(S): Rice, William G.; Schultz, Robert R.; Baker, David C.; Henderson, Louis E.

PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA; University of Tennessee Research Corp.; Rice, William G.; Schultz, Robert R.; Baker, David C.; Henderson, Louis E.

SOURCE: PCT Int. Appl., 43 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|--|----------|-----------------|------------|
| WO 9801440 | A2 | 19980115 | WO 1997-US10870 | 19970703 |
| WO 9801440 | A3 | 19980514 | | |
| W: | AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | |
| CA 2260128 | AA | 19980115 | CA 1997-2260128 | 19970703 |
| AU 9744085 | A1 | 19980202 | AU 1997-44085 | 19970703 |
| AU 737038 | B2 | 20010809 | | |
| EP 1023284 | A2 | 20000802 | EP 1997-942372 | 19970703 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | |
| US 6046228 | A | 20000404 | US 1999-214331 | 19990104 |
| PRIORITY APPLN. INFO.: | | | US 1996-21665P | P 19960705 |
| | | | WO 1997-US10870 | W 19970703 |

OTHER SOURCE(S): MARPAT 128:123799

AB Pharmaceutical compns. including a satd. 1,2-dithiaheterocyclic compd. having antiviral activity are provided. Also provided are a kit contg. the pharmaceutical compn. and methods of treating or preventing viral

disease using the compn., as well as methods for inactivating a retrovirus in a body fluid.

L17 ANSWER 35 OF 51 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1998:636937 SCISEARCH
THE GENUINE ARTICLE: 110TT
TITLE: Structural role of the 30's loop in determining the ligand specificity of the human immunodeficiency virus **protease**
AUTHOR: Swairjo M A; Towler E M; Debouck C; AbdelMeguid S S (Reprint)
CORPORATE SOURCE: SMITHKLINE BEECHAM PHARMACEUT, DEPT BIOL STRUCT, 709 SWEDELAND RD, KING OF PRUSSIA, PA 19406 (Reprint); SMITHKLINE BEECHAM PHARMACEUT, DEPT BIOL STRUCT, KING OF PRUSSIA, PA 19406; SMITHKLINE BEECHAM PHARMACEUT, DEPT MOL BIOL, KING OF PRUSSIA, PA 19406
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (4 AUG 1998) Vol. 37, No. 31, pp. 10928-10936.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 24

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The structural basis of ligand specificity in human immunodeficiency virus (HIV) **protease** has been investigated by determining the crystal structures of three chimeric HIV **proteases** complexed with SB203386, a tripeptide analogue inhibitor. The chimeras are constructed by substituting amino acid residues in the HIV type 1 (HIV-1) **protease** sequence with the corresponding residues from HIV type 2 (HIV-2) in the region spanning residues 31-37 and in the active site cavity. SB203386 is a potent **inhibitor** of HIV-1 **protease** (K-i = 18 nM) but has a decreased affinity for HIV-2 **protease** (K-i = 1280 nM). Crystallographic analysis reveals that substitution of residues 31-37 (30's loop) with those of HIV-2 **protease** renders the chimera similar to HIV-2 **protease** in both the **inhibitor** binding affinity and mode of binding (two **inhibitor** molecules per **protease** dimer). However, further substitution of active site residues 47 and 82 has a compensatory effect which restores the HIV-1-like inhibitor binding mode (one inhibitor molecule in the center of the **protease** active site) and partially restores the affinity. Comparison of the three chimeric **protease** structures with those of HIV-1 and SIV **proteases** complexed with the same inhibitor reveals structural changes in the flap regions and the 80's loops, as well as changes in the dimensions of the active site cavity. The study provides structural evidence of the role of the 30's loop in conferring **inhibitor** specificity in HIV **proteases**.

L17 ANSWER 36 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 10
ACCESSION NUMBER: 1999:25385 HCAPLUS
DOCUMENT NUMBER: 130:217538
TITLE: Dissecting the mode of action of various HIV-inhibitor classes in a stable cellular system
AUTHOR(S): Klimkait, T.; Stauffer, F.; Lupo, E.; Sonderegger-Rubli, C.

CORPORATE SOURCE: Novartis Pharma, Inc., Basel, Switz.
SOURCE: Archives of Virology (1998), 143(11), 2109-2131
CODEN: ARVIDF; ISSN: 0304-8608
PUBLISHER: Springer-Verlag Wien
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We describe a stable and sensitive HIV evaluation system, which discriminates HIV-specific membrane fusion and early transcription events and is suitable for high-throughput inhibitor screening. A human lymphocytic line, constitutively producing infectious HIV-1, serves as Env-pos. donor. A second indicator cell line carries a silent HIV-1 LTR lacZ reporter plasmid. A bicellular cocultivation setup allows titrn. and standardization of "fusion-induced gene stimulation (FIGS)" events. With few manipulations aspects of fusion and/or LTR induction can be distinguished and simultaneously assayed. Anti-Env-V3 anti-bodies prevent fusion and subsequent lacZ induction, and a Tat-specific inhibitor blocks only lacZ induction in a dose dependent manner without affecting membrane fusion. The LTR reporter is readily activated by Tat from HIV-1, HIV-2, or SIV and it responds to exogenous Tat protein. The reporter system is sensitive enough to detect single infection events on pre-seeded layers of indicator cells, which renders it potentially useful for direct virus quantification in patients' material. Moreover, our system allows to control and normalize DNA transfection efficiencies of HIV-derived plasmids. This aspect is particularly valuable for studies of RT- and **protease-inhibitors** and **resistances**, where p24 or supernatant reverse transcriptase, otherwise std. virus readouts, can be directly affected by inhibitors or mutations.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 37 OF 51 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

ACCESSION NUMBER: 1998079738 EMBASE
TITLE: Saquinavir. Clinical pharmacology and efficacy.
AUTHOR: Vella S.; Floridia M.
CORPORATE SOURCE: Dr. S. Vella, Laboratory of Virology, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Rome, Italy
SOURCE: Clinical Pharmacokinetics, (1998) 34/3 (189-201).
Refs: 56
ISSN: 0312-5963 CODEN: CPKNDH
COUNTRY: New Zealand
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
030 Pharmacology
037 Drug Literature Index
039 Pharmacy
038 Adverse Reactions Titles
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Saquinavir is an HIV **protease inhibitor** with no, or limited, effect on the activity of other structurally related human aspartic proteinases. As with other HIV **protease inhibitors**, saquinavir **inhibits** the cleavage of the gag-pol protein substrate leading to the release of structurally defective and functionally inactive viral particles. It is active on both HIV-1 and HIV-2, and also has activity on chronically infected cells and HIV strains **resistant** to reverse transcriptase inhibitors. Synergy of action has been observed with other antiretroviral drugs. Saquinavir is characterised by a low bioavailability which is further reduced in the

fasting state. Metabolism is mainly hepatic through cytochrome P450 (CYP) 3A4, but intestinal metabolism through the same system has also been reported. To achieve higher drug plasma concentrations and increase the antiviral effect, a new formulation of saquinavir with a higher bioavailability has recently been introduced. Higher plasma drug concentrations may also be obtained by combining the drug with CYP blockers, such as ritonavir or ketoconazole. Because of its metabolic interference with the CYP system, saquinavir cannot be coadministered with astemizole, terfenadine or cisapride. Rifampicin (rifampin) is also contraindicated because coadministration can lead to decreases in saquinavir concentrations. Interactions have also been reported with other drugs metabolised through the same system, including non-nucleoside reverse transcriptase **inhibitors** and HIV **protease inhibitors**. **Resistance** has been observed after both in vitro and in vivo drug exposure, with a relatively specific mutation profile compared with other **protease inhibitors**. Saquinavir is generally well tolerated, with mild gastrointestinal symptoms representing the most commonly observed adverse effects. Although characterised by low bioavailability, in phase III trials saquinavir has been shown to have clinical efficacy in terms of survival and progression rate. As with the other **protease inhibitors**, saquinavir should be used in combination with other antiretroviral drugs. Current therapeutic guidelines, however, recommend the selection of an initial treatment regimen with other **protease inhibitors** with higher in vivo activity in terms of RNA and CD4 response. The results of ongoing studies will clarify to what extent a new saquinavir formulation, recently introduced, is superior to the previous one in terms of antiviral activity and to provide comparisons with other **protease inhibitors**. Further studies are also needed to define the best place of saquinavir within treatment strategies based on **protease inhibitors**, particularly in respect to the optimal sequence for its use with other **protease inhibitors**, and the dynamics of cross-**resistance** and its role within regimens based on the combination of **protease inhibitors**.

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on STN

ACCESSION NUMBER: 1998109577 EMBASE
TITLE: Lamivudine in the management of adults with human immunodeficiency virus type 1 infection.
AUTHOR: Moyle G.J.; Gazzard B.G.
CORPORATE SOURCE: Dr. G.J. Moyle, HIV Research, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9TH, United Kingdom
SOURCE: Antiviral Therapy, (1998) 3/1 (7-18).
Refs: 66
ISSN: 1359-6535 CODEN: ANTHFA
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Lamivudine is a nucleoside analogue reverse transcriptase inhibitor of human immunodeficiency virus type 1 (HIV-1), HIV-2 and hepatitis B virus which demonstrates in vitro activity with a high therapeutic index in a range of T lymphocyte and haematopoietic precursor cell lines. It is synergistic in vitro with a range of other antiretrovirals including other nucleoside analogues, non-nucleoside reverse transcriptase

inhibitors and **protease inhibitors**, in two- and three-way combinations and is active against virus **resistant** to zidovudine and most other antiretrovirals. It exhibits excellent oral bioavailability and penetrates the CSF. In clinical use, lamivudine exhibits limited antiretroviral activity as a monotherapy, but in two- and three-drug combinations with other antiretrovirals it provides potent antiretroviral activity and significantly delays clinical events. Adverse events appear infrequent and are generally mild and self-limiting. **Resistance** to lamivudine develops rapidly in vitro and in vivo but may delay the development of zidovudine **resistance**. Dual zidovudine-lamivudine **resistance** is well documented both in vitro and in vivo although the mutation at codon 184 of reverse transcriptase characteristic of lamivudine **resistance** may, in some cases, transiently 'reverse' zidovudine **resistance** mediated through codons 215 and 41. Owing to concerns regarding selection of virus with cross-**resistance** to other nucleoside analogues (ddI, ddC and possibly abacavir), whether to use lamivudine in initial or second-line regimens is currently under debate.

L17 ANSWER 39 OF 51 MEDLINE on STN DUPLICATE 11
 ACCESSION NUMBER: 1998001419 MEDLINE
 DOCUMENT NUMBER: 98001419 PubMed ID: 9343254
 TITLE: Susceptibility of human immunodeficiency virus type 1 group O isolates to antiretroviral agents: in vitro phenotypic and genotypic analyses.
 AUTHOR: Descamps D; Collin G; Letourneur F; Apetrei C; Damond F; Loussert-Ajaka I; Simon F; Saragosti S; Brun-Vezinet F
 CORPORATE SOURCE: Laboratoire de Virologie, Hopital Bichat-Claude Bernard, Paris, France.. diane.descamps@bch-ap-hop-paris.fr
 SOURCE: JOURNAL OF VIROLOGY, (1997 Nov) 71 (11) 8893-8. Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 OTHER SOURCE: GENBANK-Y14496; GENBANK-Y14497; GENBANK-Y14498; GENBANK-Y14499; GENBANK-Y14500; GENBANK-Y14501; GENBANK-Y14502; GENBANK-Y14503; GENBANK-Y14504; GENBANK-Y14505
 ENTRY MONTH: 199711
 ENTRY DATE: Entered STN: 19971224
 Last Updated on STN: 19990129
 Entered Medline: 19971113

AB We investigated the phenotypic and genotypic susceptibility of 11 human immunodeficiency virus type 1 (HIV-1) group O strains to nucleoside and nonnucleoside reverse transcriptase (RT) **inhibitors** and **protease inhibitors** in vitro. Phenotypic susceptibility was determined by using a standardized in vitro assay of RT inhibition, taking into account the replication kinetics of each strain. HIV-1 group M and HIV-2 isolates were used as references. DNA from cocultured peripheral blood mononuclear cells was amplified by using pol-specific group O primers and cloned for sequencing. Group O isolates were highly sensitive to nucleoside inhibitors, but six isolates were naturally highly **resistant** to all of the nonnucleoside RT inhibitors tested. Phylogenetic analysis of the pol gene showed that these isolates formed a separate cluster within group O, and genotypic analysis revealed a tyrosine-to-cysteine substitution at residue 181. Differences in susceptibility to saquinavir and ritonavir (RTV) were not

significant between group O and group M isolates, although the 50% inhibitory concentration of RTV for group O isolates was higher than that for the HIV-1 subtype B strains. The study of HIV-1 group O susceptibility to antiretroviral drugs revealed that the viruses tested had specific phenotypic characteristics contrasting with the group M phenotypic expression.

L17 ANSWER 40 OF 51 MEDLINE on STN DUPLICATE 12
 ACCESSION NUMBER: 97291239 MEDLINE
 DOCUMENT NUMBER: 97291239 PubMed ID: 9145853
 TITLE: Antiviral properties of palinavir, a potent inhibitor of the human immunodeficiency virus type 1 **protease**.
 AUTHOR: Lamarre D; Croteau G; Wardrop E; Bourgon L; Thibeault D; Clouette C; Vaillancourt M; Cohen E; Pargellis C; Yoakim C; Anderson P C
 CORPORATE SOURCE: Department of Biochemistry, Bio-Mega Research Division of Boehringer Ingelheim (Canada) Ltd., Laval, Quebec.
 SOURCE: ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, (1997 May) 41 (5) 965-71.
 Journal code: 0315061. ISSN: 0066-4804.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199708
 ENTRY DATE: Entered STN: 19970902
 Last Updated on STN: 19990129
 Entered Medline: 19970821

AB Palinavir is a potent inhibitor of the human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) **proteases**. Replication of laboratory strains (HIV-1, HIV-2, and simian immunodeficiency virus) and HIV-1 clinical isolates is inhibited by palinavir with 50% effective concentrations ranging from 0.5 to 30 nM. The average cytotoxic concentration of palinavir (35 microM) in the various target cells indicates a favorable therapeutic index. Potent antiviral activity is retained with increased doses of virus and with clinical isolates **resistant** to zidovudine (AZT), didanosine (ddI), or nevirapine. Combinations of palinavir with either AZT, ddI, or nevirapine demonstrate synergy or additivity in the inhibition of HIV-1 replication. Palinavir retains anti-HIV-1 activity when administered postinfection until times subsequent to the reverse transcription step. In chronically infected CR-10 cells, palinavir blocks Gag precursor polyprotein processing completely, reducing greater than 99% of infectious particle production. The results indicate that the antiviral activity of palinavir is specific to **inhibition** of the viral **protease** and occurs at a late stage in the replicative cycle of HIV-1. On the basis of the potent in vitro activity, low-level cytotoxicity, and other data, palinavir was selected for in-depth preclinical evaluation.

L17 ANSWER 41 OF 51 MEDLINE on STN
 ACCESSION NUMBER: 1998111714 MEDLINE
 DOCUMENT NUMBER: 98111714 PubMed ID: 9450061
 TITLE: Characterisation of drug **resistant** retroviral proteinase mutants.
 AUTHOR: Good V M; Anderson M M; Baker J J; Moloudi A; James C H; Wilderspin A F
 CORPORATE SOURCE: Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, London, UK.

Stucker 09/980,777

.SOURCE: BIOCHEMICAL SOCIETY TRANSACTIONS, (1997 Nov) 25 (4) S633.
Journal code: 7506897. ISSN: 0300-5127.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980422
Last Updated on STN: 20000303
Entered Medline: 19980413

L17 ANSWER 42 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 13

ACCESSION NUMBER: 1997:228825 HCAPLUS
DOCUMENT NUMBER: 126:301431
TITLE: New drugs - Reports of new drugs recently approved by
the FDA: ritonavir
AUTHOR(S): Ohta, Yukari; Shinkai, Ichiro
CORPORATE SOURCE: Banyu Clinical Research, Tokyo, Japan
SOURCE: Bioorganic & Medicinal Chemistry (1997), 5(3), 461-462
CODEN: BMECEP; ISSN: 0968 0896
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Ritonavir (Norvir, A 84538, or ABT 538) is a peptidomimetic inhibitor of both HIV-1 and **HIV-2 proteases**. The concn. of drug that inhibits 50% of viral replication (EC50) ranged from 3.8 to 153 nM depending upon the HIV-1 isolate and the cells employed. The av. EC50 for low passage clin. isolates was 22 nM. In a 1090-patient study, 1.2 g of the drug used concomitantly with existing nucleoside therapy, produced a significant decrease in mean viral RNA levels of placebo and an increase in av. change of CD4 count over the first 16 wk. After seven months the mortality rate was 4.8% for ritonavir patients and 8.4% for placebo. Ritonavir demonstrated additive effects against HIV-1 in combination with either zidovudine (ZDV) or didanosine (ddI). Genotypic anal. of HIV-1 isolates with reduced susceptibility to ritonavir showed mutations in the HIV **protease** gene at amino acid positions 84 (Ile to Val), 82 (Val to Phe), 71 (Ala to Val), and 46 (Met to Ile). Phenotypic and genotypic changes in HIV isolates from selected patients treated with ritonavir were monitored in phase I/II trials over a period of 3-32 wk. Mutation appeared to occur in a stepwise and ordered fashion. The potential for HIV cross-**resistance** between **protease inhibitors** has not been fully explored. The abs. bioavailability of ritonavir has not been detd. After a 600 mg dose of oral soln., peak concns. of ritonavir were achieved approx. 2 and 4 h after dosing under fasting and nonfasting conditions, resp. The isopropylthiazole oxidn. metabolite (M-2) is the major metabolite. Studies utilizing human liver microsome have demonstrated that cytochrome P 450 3A (CYP3A) is the major isoform involved in ritonavir metab., although CYP2D6 also contributes to the formation of M-2. Agents that increase CYP3A activity would be expected to increase the clearance of ritonavir resulting in decrease of ritonavir plasma concn. Ritonavir can produce a large increase in plasma concns. of certain highly metabolized drugs. Ritonavir prevents fast metab. of saquinavir allowing increased blood levels. Addn. of saquinavir is not expected to accelerate **resistance** to ritonavir due to the distinct mutation profiles of both drugs. Norvir capsules are available for oral administration in a strength of 100 mg ritonavir. Norvir oral soln. is also available for oral administration as 80 mg/mL of ritonavir in a flavored vehicle.

L17 ANSWER 43 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 14
ACCESSION NUMBER: 1997:98389 HCAPLUS
DOCUMENT NUMBER: 126:194888
TITLE: SRR-SB3, a disulfide-containing macrolide that inhibits a late stage of the replicative cycle of human immunodeficiency virus
AUTHOR(S): Witvrouw, M.; Balzarini, J.; Pannecouque, C.; Jhaumeer-Laulloo, S.; Este, J. A.; Schols, D.; Cherepanov, P.; Schmit, J.-C.; Debyser, Z.; Vandamme, A.-M.; Desmyter, J.; Ramadas, S. R.; De Clercq, E.
CORPORATE SOURCE: Rega Inst. Med. Res., Katholieke Univ. Leuven, Louvain, B-3000, Belg.
SOURCE: Antimicrobial Agents and Chemotherapy (1997), 41(2), 262-268
CODEN: AMACCQ; ISSN: 0066-4804
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB From a series of macrocyclin diamides possessing the disulfide linkage, only SRR-SB3, a compd. that complexes with zinc, was found to inhibit human immunodeficiency virus type 1 (HIV-1; strain IIIB) replication at a concn. of 1.8 to 6.5 .mu.g/mL in MT-4, CEM, and peripheral blood mononuclear cells. SRR-SB3 was toxic to MT-4 cells at a concn. of 15.9 .mu.g/mL, resulting in a selectivity index of 9 in these cells. This macrolide was also effective against various other HIV-1 strains, including clin. isolates and HIV-1 strains **resistant** to **protease inhibitors** and nucleoside and nonnucleoside reverse transcriptase inhibitors. It was also active against various HIV-2 strains, simian immunodeficiency virus (strain MAC251), and Moloney murine sarcoma virus, but not against viruses other than retroviruses. In addn., the compd. was found to inhibit chronic HIV-1 infections in vitro. The compd. in combination with other antiviral agents, such as zidovudine, zalcitabine, and stavudine, showed an effect that was between additive and synergistic. Time-of-addn. expts. indicated that SRR-SB3 acts at a late stage of the HIV-1 replicative cycle.

L17 ANSWER 44 OF 51 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:113757 BIOSIS
DOCUMENT NUMBER: PREV199800113757
TITLE: Treatment with **protease** inhibitors in HIV -2 infected patients.
AUTHOR(S): Matheron, S. (1); Simon, F.; Lepretre, A.; Pueyo, S.; **Brun-Vezinet, F.**
CORPORATE SOURCE: (1) Bichat Hosp., 46 rue Henri Huchard, Paris 75018 France
SOURCE: Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (1997) Vol. 37, pp. 261. Meeting Info.: 37th Interscience Conference on Antimicrobial Agents and Chemotherapy Toronto, Ontario, Canada September 28-October 1, 1997 ICAAC
DOCUMENT TYPE: Conference
LANGUAGE: English

L17 ANSWER 45 OF 51 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1998014166 EMBASE
TITLE: The role of stavudine in the management of adults with HIV infection.
AUTHOR: Moyle G.J.; Gazzard B.G.

Stucker 09/980,777

CORPORATE SOURCE: G.J. Moyle, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, United Kingdom
SOURCE: Antiviral Therapy, (1997) 2/4 (207-218).

Refs: 72

ISSN: 1359-6535 CODEN: ANTHFA

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; General Review

FILE SEGMENT: 004 Microbiology
017 Public Health, Social Medicine and Epidemiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
038 Adverse Reactions Titles

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Combinations of two nucleoside analogue reverse transcriptase inhibitors plus a third agent represent the current standard for antiretroviral therapy. Stavudine is a nucleoside analogue that demonstrates in vitro activity against human immunodeficiency virus type 1 (HIV-1) and HIV-2 within an acceptable therapeutic index in a range of T lymphocyte and haematopoietic precursor cell lines. It is additive or synergistic in vitro with a number of other antiretrovirals including **protease inhibitors** in two and three way combinations and is active against zidovudine-resistant virus. It exhibits excellent oral bioavailability, with CSF penetration. In clinical use, stavudine exhibits antiretroviral activity as a monotherapy similar to zidovudine, and is of proven clinical benefit in zidovudine-pretreated patients. In combination with didanosine and/or nelfinavir it results in more substantial and durable responses in immunological and virological markers than reported with either drug alone. Comparative trials in zidovudine-experienced patients suggest a similar frequency of adverse events to that observed with zidovudine. Peripheral neuropathy is the most common dose-limiting toxicity with haematological and hepatic function disturbance being infrequent. Reasons for stavudine failure are not established, with no consistent genotypic pattern being associated with changes in stavudine sensitivity in vitro or in vivo. The role of stavudine is as a component of triple therapy regimens both in initial therapy and in patients with prior zidovudine experience.

L17 ANSWER 46 OF 51 MEDLINE on STN

ACCESSION NUMBER: 97037995 MEDLINE

DOCUMENT NUMBER: 97037995 PubMed ID: 8883623

TITLE: HIV **protease inhibitors**.

AUTHOR: Carr A; Cooper D A

CORPORATE SOURCE: HIV Medicine Unit, St Vincent's Hospital, Sydney, Australia.

SOURCE: AIDS, (1996) 10 Suppl A S151-7. Ref: 51
Journal code: 8710219. ISSN: 0269-9370.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General review; (REVIEW)
(REVIEW, TUTORIAL)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 199701

ENTRY DATE: Entered STN: 19970219

Last Updated on STN: 20000303

Entered Medline: 19970122

L17 ANSWER 47 OF 51 MEDLINE on STN

DUPLICATE 15

ACCESSION NUMBER: 95014470 MEDLINE
DOCUMENT NUMBER: 95014470 PubMed ID: 7929352
TITLE: Crystal structure at 1.9-A resolution of human immunodeficiency virus (HIV) II **protease** complexed with L-735,524, an orally bioavailable **inhibitor** of the HIV **proteases**.
AUTHOR: Chen Z; Li Y; Chen E; Hall D L; Darke P L; Culberson C; Shafer J A; Kuo L C
CORPORATE SOURCE: Department of Biological Chemistry, Merck Research Laboratories, West Point, Pennsylvania 19486.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Oct 21) 269 (42) 26344-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 20000303
Entered Medline: 19941122

AB L-735,524 is a potent, orally bioavailable inhibitor of human immunodeficiency virus (HIV) **protease** currently in a Phase II clinical trial. We report here the three-dimensional structure of L-735,524 complexed to **HIV-2 protease** at 1.9-A resolution, as well as the structure of the native **HIV-2 protease** at 2.5-A resolution. The structure of **HIV-2 protease** is found to be essentially identical to that of HIV-1 **protease**. In the crystal lattice of the **HIV-2 protease** complexed with L-735,524, the inhibitor is chelated to the active site of the homodimeric enzyme in one orientation. This feature allows an unambiguous assignment of protein-ligand interactions from the electron density map. Both Fourier and difference Fourier maps reveal clearly the closure of the flap domains of the **protease** upon L-735,524 binding. Specific interactions between the enzyme and the inhibitor include the hydroxy group of the hydroxyaminopentane amide moiety of L-735,524 ligating to the carboxyl groups of the essential Asp 25 and Asp-25' enzymic residues and the amide oxygens of the inhibitor hydrogen bonding to the backbone amide nitrogen of Ile-50 and Ile-50' via an intervening water molecule. A second bridging water molecule is found between the amide nitrogen N2 of L-735,524 and the carboxyl oxygen of Asp-29'. Although other hydrogen bonds also add to binding, an equally significant contribution to affinity arises from hydrophobic interactions between the **protease** and the **inhibitor** throughout the pseudo-symmetric S1/S1', S2/S2', and S3/S3' regions of the enzyme. Except for its pyridine ring, all lipophilic moieties (t-butyl, indanyl, benzyl, and piperidyl) of L-735,524 are rigidly defined in the active site.

L17 ANSWER 48 OF 51 MEDLINE on STN DUPLICATE 16
ACCESSION NUMBER: 94162203 MEDLINE
DOCUMENT NUMBER: 94162203 PubMed ID: 8117657
TITLE: Human immunodeficiency virus type 1 **protease inhibitors**: evaluation of **resistance** engendered by amino acid substitutions in the enzyme's substrate binding site.
AUTHOR: Sardana V V; Schlabach A J; Graham P; Bush B L; Condra J H; Culberson J C; Gotlib L; Graham D J; Kohl N E; LaFemina R

L; +
CORPORATE SOURCE: Department of Virus and Cell Biology, Merck Research
Laboratories, West Point, Pennsylvania 19486.
SOURCE: BIOCHEMISTRY, (1994 Mar 1) 33 (8) 2004-10.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940412
Last Updated on STN: 19960129
Entered Medline: 19940404

AB The human immunodeficiency virus type 1 (HIV-1) **protease** is a homodimeric aspartyl endopeptidase that is required for virus replication. A number of specific, active-site inhibitors for this enzyme have been described. Many of the inhibitors exhibit significant differences in activity against the HIV-1 and HIV type 2 (HIV-2) enzymes. An initial study was conducted to ascertain the HIV-1 **protease's** potential to lose sensitivity to several test inhibitors while retaining full enzymatic activity. The substrate binding sites of the HIV-1 and HIV-2 enzymes are almost fully conserved, except for four amino acid residues at positions 32, 47, 76, and 82. Accordingly, recombinant mutant type 1 **proteases** were constructed that contained the cognate type 2 residue at each of these four positions. The substitution at position 32 resulted in a significant adverse effect on inhibitor potency. However, this substitution also mediated a noted increase in the K_m of the substrate. Individual substitutions at the remaining three positions, as well as a combination of all four substitutions, had very little effect on enzyme activity or inhibitor susceptibility. Hence, the four studied active site residues are insufficient to be responsible for differences in inhibitor sensitivity between the HIV-1 and HIV-2 **proteases** and are unlikely to contribute to the generation of inhibitor-resistant mutant HIV-1 **protease**.

L17 ANSWER 49 OF 51 MEDLINE on STN
ACCESSION NUMBER: 95157322 MEDLINE
DOCUMENT NUMBER: 95157322 PubMed ID: 7854190
TITLE: Qualitative study of drug **resistance** in retroviral **protease** using structural modeling and site-directed mutagenesis.
AUTHOR: Culberson J C; Bush B L; Sardana V V
CORPORATE SOURCE: Department of Molecular Systems, Merck Research Laboratories, West Point, Pennsylvania 19486.
SOURCE: METHODS IN ENZYMOLOGY, (1994) 241 385-94. Ref: 40
Journal code: 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 20000303
Entered Medline: 19950313

L17 ANSWER 50 OF 51 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

Stucker 09/980,777

ACCESSION NUMBER: 93357869 EMBASE
DOCUMENT NUMBER: 1993357869
TITLE: In vitro anti-human immunodeficiency virus (HIV) activity
of XM323, a novel HIV **protease inhibitor**
AUTHOR: Otto M.J.; Reid C.D.; Garber S.; Lam P.Y.-S.; Scarnati H.;
Bacheler L.T.; Rayner M.M.; Winslow D.L.
CORPORATE SOURCE: Du Pont Merck Pharmaceutical Company, Glenolden, PA 19036,
United States
SOURCE: Antimicrobial Agents and Chemotherapy, (1993) 37/12
(2606-2611).
ISSN: 0066-4804 CODEN: AMACCO
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB XM323 represents a novel class of potent inhibitors of human immunodeficiency virus (HIV) **protease**. In vitro studies have shown that inhibition of this enzyme translates into potent inhibition of replication of HIV type 1 (HIV-1) and HIV-2. The inhibition of virus replication was assessed with three assays designed to measure the production of infectious virus, viral RNA, or p24 antigen. The production of mature infectious virions was measured with a yield reduction assay. By this assay, several strains and isolates of HIV-1 and HIV-2 were shown to be susceptible to XM323 in two lymphoid cell lines (MT-2 and H9) and in normal peripheral blood mononuclear cells, with a concentration required for 90% inhibition (IC90) of 0.12 \pm 0.04 μ M (mean \pm standard deviation). The production of HIV-1(RF) RNA was measured with an RNA hybridization-capture assay. With this assay, XM323 was shown to be a potent inhibitor of HIV-1(RF) replication, with an IC90 of 0.063 \pm 0.032 μ M. A third measure of virus replication, the production of p24 viral antigen, an essential protein component of the virion, was determined with the AIDS Clinical Trial Group-Department of Defense peripheral blood mononuclear cell consensus assay. This assay was used for expanded testing of XM323 against 28 clinical isolates and laboratory strains of HIV-1. XM323 was shown to be equally effective against zidovudine-susceptible and zidovudine-**resistant** isolates of HIV-1, with an overall IC90 of 0.16 \pm 0.06 μ M.

L17 ANSWER 51 OF 51 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1994:94830 HCAPLUS
DOCUMENT NUMBER: 120:94830
TITLE: CGP 53437, an orally bioavailable inhibitor of human immunodeficiency virus type 1 **protease** with potent antiviral activity
AUTHOR(S): Alteri, E.; Bold, G.; Cozens, R.; Faessler, A.; Klimkait, T.; Lang, M.; Lazdins, J.; Poncioni, B.; Roesel, J. L.; et al.
CORPORATE SOURCE: Pharm. Div., Ciba-Geigy Ltd., Basel, CH-4002, Switz.
SOURCE: Antimicrobial Agents and Chemotherapy (1993), 37(10), 2087-92
CODEN: AMACCO; ISSN: 0066-4804
DOCUMENT TYPE: Journal
LANGUAGE: English
AB CGP 53437 is a peptidomimetic inhibitor of human immunodeficiency virus type 1 proteinase contg. a hydroxyethylene isostere. The compd. inhibited

recombinant human immunodeficiency virus-1 proteinase with a K_i of 0.2 nM. The inhibition const. vs. human cathepsin D and human cathepsin E was 4 nM. Human pepsin and gastricsin were inhibited with K_i s of 8 and 500 nM, resp., and human renin was inhibited with a K_i of 190 μ M. The replication of human immunodeficiency virus-1/LAV, human immunodeficiency virus-1/Z-84, and human immunodeficiency virus-1/pLAI was inhibited with a 90% ED of 0.1 μ M in acutely infected MT-2 cells. The 50% cytotoxic dose was 100 μ M. Similar antiviral activity was obsd. when the compd. was added up to 10 h after infection. At the effective concn., processing of Gag precursor protein p55 was greatly reduced, confirming an action on the late stage of the virus life cycle, as expected. The efficacy of the inhibitor was also demonstrated by using primary human peripheral blood lymphocytes infected with the human immunodeficiency virus-1/LAV strain, low-passage clin. isolates obtained from human immunodeficiency virus-1-seropos. individuals (including a zidovudine-resistant strain), and **human immunodeficiency virus-2/ROD**. In these cells, CGP 53437 delayed the onset of human immunodeficiency virus replication in a dose-dependent fashion (substantial effects with concns. of $\geq 0.1 \mu$ M) as long as the inhibitor was maintained in the culture. CGP 53437 was orally bioavailable in mice. Concns. in plasma 10-fold in excess of the in vitro antiviral 90% ED could be sustained for several h after oral application of 120 mg/kg. Therefore, CGP 53437 has the potential to be a therapeutically useful anti-human immunodeficiency virus agent for the treatment of AIDS.

=> d his

(FILE 'HOME' ENTERED AT 13:54:00 ON 10 OCT 2003)

FILE 'MEDLINE' ENTERED AT 13:54:09 ON 10 OCT 2003

L1 7717 S HIV? AND PROTEASE
L2 1701 S L1 AND (RESISTANCE OR RESISITENCE)
L3 517 S L2 AND MUTATION
L4 75 S L3 AND (90 OR L90M)
L5 31 S L4 AND PY<=2000
L6 4 S HIV1 AND HIV2 AND PROTEASE
L7 3023 S HIV-1 AND HIV-2
L8 175 S L7 AND PROTEASE
L9 40 S L8 AND (SIMILAR OR IDENTITY OR HOMOLOGY OR CORRESPOND)

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF

LOGOFF? (Y)/N/HOLD:y

COST IN U.S. DOLLARS

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| FULL ESTIMATED COST | 35.64 | 35.85 |
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STN INTERNATIONAL LOGOFF AT 14:44:12 ON 10 OCT 2003

Clinical cross-resistance between the HIV-1 protease inhibitors saquinavir and indinavir and correlations with genotypic mutations

Jonathan M. Schapiro, Mark A. Winters, Jody Lawrence
and Thomas C. Merigan

Objectives: To determine the clinical efficacy of the HIV-1 protease inhibitor indinavir (IDV) in saquinavir (SQV)-experienced patients and delineate the developing drug-resistance patterns.

Design: Open-label prospective clinical trial.

Setting: University hospital research center.

Patients: Ten patients who had completed a SQV monotherapy study in which they had received SQV at a dose of 3600 or 7200 mg daily (two and fourfold the standard dose).

Interventions: At enrollment patients received IDV for 4 weeks as monotherapy, after which zidovudine (ZDV) and lamivudine (3TC) were added to their drug regimen. Patients then received combination therapy (IDV-ZDV-3TC) for an additional 20 weeks to complete a total of 24 weeks of therapy.

Main outcome measures: Plasma HIV RNA viral load and CD4+ T-cell counts were monitored. Sequencing of the HIV protease gene was performed to determine the development of resistance mutations. Plasma samples for sequencing were taken before initial SQV therapy, after SQV therapy before starting IDV, and after 24 weeks of IDV therapy.

Results: The average duration of high-dose SQV before starting IDV was 58 ± 29.2 weeks. A $0.58 \log_{10}$ RNA copies/ml increase was noted during the 3-week washout phase followed by a mean reduction in plasma HIV RNA viral load of $1.2 \log_{10}$ RNA copies/ml after 4 weeks of IDV. After the addition of ZDV and 3TC at week 4, HIV RNA continued to fall reaching a mean reduction of $1.96 \log_{10}$ RNA copies/ml at week 24. Plasma HIV RNA was below 400 RNA copies/ml in six out of nine patients at week 24. CD4+ T-cell counts showed a gradual rise from $328 \times 10^6/l$ to $453 \times 10^6/l$ by week 24. SQV therapy had resulted in multiple mutations in the protease gene. Six of the patients had developed five or more mutations: L90M in two, G48V in four (of which three also contained L10I), and V82A in three. Patients in whom plasma HIV RNA was not durably suppressed by subsequent IDV combination therapy developed multiple (up to four) additional mutations within 24 weeks, including codons 54, 82 and 93 amongst others. No clear correlation was found between the mutations that had developed in individual patients after SQV and the subsequent efficacy of IDV.

Conclusion: Prolonged use of SQV at potent doses in the presence of elevated viral load levels resulted in the development of multiple resistance mutations. Individual resistance patterns varied greatly between patients, as did their virological response to therapy. Resistance assays may be useful in identifying which patients will benefit from salvage therapy with a second protease inhibitor. © 1999 Lippincott Williams & Wilkins

AIDS 1999, 13:359–365

Keywords: HIV, protease inhibitors, antiretroviral therapy, saquinavir, indinavir, resistance mutations, cross-resistance

From the Center for AIDS Research, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California, USA.

Sponsorship: Support for this study was provided by Roche Pharmaceuticals, Inc., Nutley, New Jersey.

Requests for reprints to: Dr Thomas C. Merigan, Center for AIDS Research, S-156 Grant Building, Stanford University School of Medicine, Stanford, CA 94305, USA.

Date of receipt: 18 August 1998; revised: 12 November 1998; accepted: 18 November 1998.

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Introduction

HIV-1 protease inhibitors produce significant improvements in both surrogate markers of HIV infection such as plasma HIV RNA viral load and CD4+ T-cell counts, and clinical outcomes such as time to diagnosis of AIDS or death [1-6]. Many patients treated with combination antiretroviral drug regimens containing protease inhibitors show a suppression of plasma HIV RNA viral load to undetectable levels for prolonged periods of time, the ultimate duration of which remains to be determined [7-10]. These impressive results are not seen in all treated patients. Incomplete and transient reductions in viral load are seen in many patients with the return of high plasma levels of HIV often containing resistance mutations to the administered protease inhibitor [11-15]. More advanced disease, syncytium-inducing phenotype and poor patient compliance with drug-taking are some of the factors that appear to be associated with a less favorable response [16-18]. Rational therapeutic options are needed for patients who have failed therapy with a particular protease inhibitor.

Characteristic mutational patterns in the HIV protease gene following therapy with the individual protease inhibitors have been determined and a strategy for the utility of one drug following the failure of the other has been suggested based on the degree of overlapping of these mutational patterns [19-21]. The ability of these mutational patterns to predict clinical cross-resistance and the actual degree of clinically relevant cross-resistance between the protease inhibitors has yet to be determined. These clinical data are urgently needed to help guide our decisions regarding therapy in drug-experienced patients and possibly in our decision regarding the choice of initial therapy. Some data has suggested that patients who have failed therapy with saquinavir (SQV) may still benefit from therapy with indinavir (IDV) [21]. We therefore conducted a pilot study to determine the clinical efficacy of IDV in high dose (3600 or 7200 mg daily) SQV-experienced patients and attempted to determine the ability of baseline mutations to predict the degree of clinical benefit.

Methods

Patients

The study included 10 patients who had completed a SQV monotherapy study in which patients had received SQV hard-gel capsules at a dose of 3600 or 7200 mg daily in six divided doses (two and fourfold the standard dose) [4]. Patients were required to have received 6 months or more of such SQV therapy and to be currently receiving drug or to have discontinued it within 6 months of enrollment. Patients could not

have received therapy with IDV, ritonavir or any investigational protease inhibitors, but previous reverse transcriptase inhibitor (RTI) therapy was allowed. Patients were required to have evidence of incomplete suppression of viral load with baseline plasma HIV RNA levels above 5000 RNA copies/ml on their current regimen, which was combination therapy in six patients and monotherapy in four patients. There were no restrictions on baseline CD4+ T-cell counts.

Therapy

All antiretroviral therapy including SQV and any RTI were discontinued 3 weeks prior to enrollment for a brief washout phase. At enrollment patients received IDV 800 mg three times daily for 4 weeks as monotherapy, after which zidovudine (ZDV) 200 mg three times daily and lamivudine (3TC) 150 mg twice daily were added to their drug regimen. Patients then received the IDV-ZDV-3TC combination therapy for an additional 20 weeks to complete a total of 24 weeks of therapy. Patients demonstrating ZDV toxicity were switched to stavudine 40 mg twice daily in combination with 3TC and IDV. Study enrollment was discontinued after 10 patients, because data that were becoming available raised concerns regarding even a brief washout and monotherapy study phase.

Measurements

Patients were evaluated for evidence of clinical toxicity at weeks 2, 4, 8, 12, 16, 20, 24 and 28. In addition, safety laboratory tests including a complete blood count with differential, platelets, chemistry, liver function tests and urinalysis were performed at enrollment and at the above specified times. Plasma HIV RNA viral load was determined at the following times: when discontinuing all drugs 3 weeks prior to enrollment, at enrollment when starting IDV monotherapy (baseline) and at weeks 2, 4, 8, 12, 16, 20 and 24. Peripheral blood mononuclear cell (PBMC) proviral DNA levels and CD4+ T-cell counts were also determined at these timepoints. Population-based sequencing of the HIV protease gene was performed on plasma samples from baseline and week 24 to determine the appearance of resistance mutations. In addition, we had previously performed similar sequencing of samples taken from these patients before they began the SQV monotherapy study and after the first 24 weeks of SQV monotherapy.

Plasma HIV RNA

Plasma HIV RNA levels were determined using a previously described reverse transcriptase (RT) polymerase chain reaction (PCR) technique [22], which was validated in a multicenter study [23]. Duplicate samples of plasma were ultracentrifuged, and the pellets were extracted using phenol-chloroform. The resulting RNA pellets were reverse transcribed along with a standard curve of known RNA copy number, and then amplified by PCR with *gag*-specific primers. The amount of product in each reaction was measured using

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a non-isotopic enzyme hybridization assay and expressed as optical density. The standard curve was generated by plotting RNA copy number against optical density, and the equation that describes the curve was used to calculate the RNA copy number of the patient samples (expressed in \log_{10} copies/ml plasma).

PBMC proviral DNA

PBMC viral DNA levels were determined using a previously described quantitative PCR technique [24]. Aliquots of 1×10^6 PBMC pellets were lysed with proteinase K, and 250 000 cell equivalents were amplified in duplicate with a standard curve of known DNA copy number. The amount of product in each reaction was measured using a non-isotopic enzyme hybridization assay and expressed as optical density. The standard curve was generated by plotting DNA copy number against optical density, and the equation that describes the curve was used to calculate the DNA copy number of the patient samples, which was corrected for CD4 percentage and expressed in \log_{10} DNA copies/ 10^6 CD4 cells.

CD4+ T-cell counts

CD4 + T-cell counts were determined by the Stanford University Hospital AIDS Clinical Trials Group-qualified flow cytometry laboratory.

Sequencing the protease gene

Complete sequencing of the protease gene was performed by standard dye-terminator sequencing. Cryopreserved plasma was extracted as previously described [22], and reverse-transcribed using primer Pro1 (TGGAGTATTGTATGGATTTTCAG). The cDNA was then amplified by PCR under standard conditions using primer Pro2 (CAGAGCCAACAGC CCCACCA). The first-round PCR product was used to generate a second PCR fragment using primers for-PRO4 (TGTAACGACGGCCAGTAGCCC CACCAGAAGAGAGCTT), which contains the M13(-20) primer sequence, and primer IR. This product was sequenced using the M13 forward primer and dye-labeled dideoxy-terminators using an Applied

Biosystems Model 370A (Foster City, California, USA). All sequences were proofread manually.

Results

Patients

Ten patients were enrolled in the study. The mean baseline plasma HIV RNA levels were $4.48 \pm 0.60 \log_{10}$ copies/ml. Mean plasma RNA levels before washout were $3.90 \pm 0.80 \log_{10}$ copies/ml. Baseline CD4+ T-cell counts were also collected both before and after washout from previous therapy, but in a number of patients one of the two measurements was not available. CD4+ T-cell counts were $425 \pm 170 \times 10^6/l$ before and $328 \pm 132 \times 10^6/l$ after washout and before starting IDV. Five patients had originally received SQV monotherapy at a dose of 3600 mg daily, and five patients at a dose of 7200 mg daily (Table 1). The average duration of high-dose SQV was 58 ± 29.2 weeks. In addition, patients had received standard dose SQV for variable periods of time and six patients had also received therapy with RTI. One patient started all three drugs together; therefore, his data was not analyzed for the monotherapy period and was only included for weeks 8–12. One patient discontinued study medication at week 8 due to non-compliance and his data was included until that point.

Plasma HIV RNA viral load

During the 3-week washout period, during which patients discontinued their prestudy therapy, a mean rise of $0.58 \pm 0.46 \log_{10}$ RNA copies/ml was noted, which was followed by a sharp drop in viral load following the initiation of IDV monotherapy (Fig. 1a). The mean reduction in plasma HIV RNA viral load was $0.87 \pm 0.48 \log_{10}$ copies/ml at week 2, and $1.2 \pm 0.57 \log_{10}$ copies/ml at week 4. Following the addition of ZDV and 3TC at week 4, HIV RNA viral load continued to drop, remaining $1.96 \pm 0.68 \log_{10}$ copies/ml below baseline at week 24. Plasma HIV

Table 1. Treatment History of Saquinavir-treated Patients Switched to Indinavir

| Patient | Saquinavir Monotherapy | Follow-Up Therapy Prior to Indinavir Study |
|---------|---------------------------------|---|
| A | SQV (3600 mg/day) for 24 weeks | SQV (3600 mg/day) + ZDV for 24 weeks; then SQV (1800 mg/day) + ZDV for 56 weeks |
| B | SQV (7200 mg/day) for 24 weeks | 3TC + d4T for 28 weeks |
| C | SQV (7200 mg/day) for 100 weeks | n/a |
| D | SQV (3600 mg/day) for 24 weeks | SQV (1800 mg/day) for 80 weeks |
| E | SQV (3600 mg/day) for 72 weeks | no therapy for 24 weeks |
| F | SQV (7200 mg/day) for 48 weeks | SQV (1800 mg/day) for 4 weeks; then SQV (1800 mg/day) + ZDV + 3TC for 20 weeks |
| G | SQV (3600 mg/day) for 48 weeks | no therapy for 24 weeks; then ZDV + 3TC for 24 weeks |
| H | SQV (7200 mg/day) for 92 weeks | n/a |
| I | SQV (3600 mg/day) for 88 weeks | SQV (3600 mg/day) + ZDV + 3TC for 20 weeks |
| J | SQV (7200 mg/day) for 60 weeks | SQV (1800 mg/day) + ZDV + 3TC for 12 weeks; then ZDV + 3TC for 20 weeks |

n/a = non-applicable; patients stayed on their original saquinavir regimen without modification

a non-isotopic enzyme hybridization assay and expressed as optical density. The standard curve was generated by plotting RNA copy number against optical density, and the equation that describes the curve was used to calculate the RNA copy number of the patient samples (expressed in \log_{10} copies/ml plasma).

PBMC proviral DNA

PBMC viral DNA levels were determined using a previously described quantitative PCR technique [24]. Aliquots of 1×10^6 PBMC pellets were lysed with proteinase K, and 250 000 cell equivalents were amplified in duplicate with a standard curve of known DNA copy number. The amount of product in each reaction was measured using a non-isotopic enzyme hybridization assay and expressed as optical density. The standard curve was generated by plotting DNA copy number against optical density, and the equation that describes the curve was used to calculate the DNA copy number of the patient samples, which was corrected for CD4 percentage and expressed in \log_{10} DNA copies/ 10^6 CD4 cells.

CD4+ T-cell counts

CD4 + T-cell counts were determined by the Stanford University Hospital AIDS Clinical Trials Group-qualified flow cytometry laboratory.

Sequencing the protease gene

Complete sequencing of the protease gene was performed by standard dye-terminator sequencing. Cryopreserved plasma was extracted as previously described [22], and reverse-transcribed using primer Pro1 (TGGAGTATTGTATGGATTTTCAG). The cDNA was then amplified by PCR under standard conditions using primer Pro2 (CAGAGCCAACAGC CCCACCA). The first-round PCR product was used to generate a second PCR fragment using primers for-PRO4 (TGTAACGACGGCCAGTAGCCC CACCAGAAGAGAGCTT), which contains the M13(-20) primer sequence, and primer IR. This product was sequenced using the M13 forward primer and dye-labeled dideoxy-terminators using an Applied

Biosystems Model 370A (Foster City, California, USA). All sequences were proofread manually.

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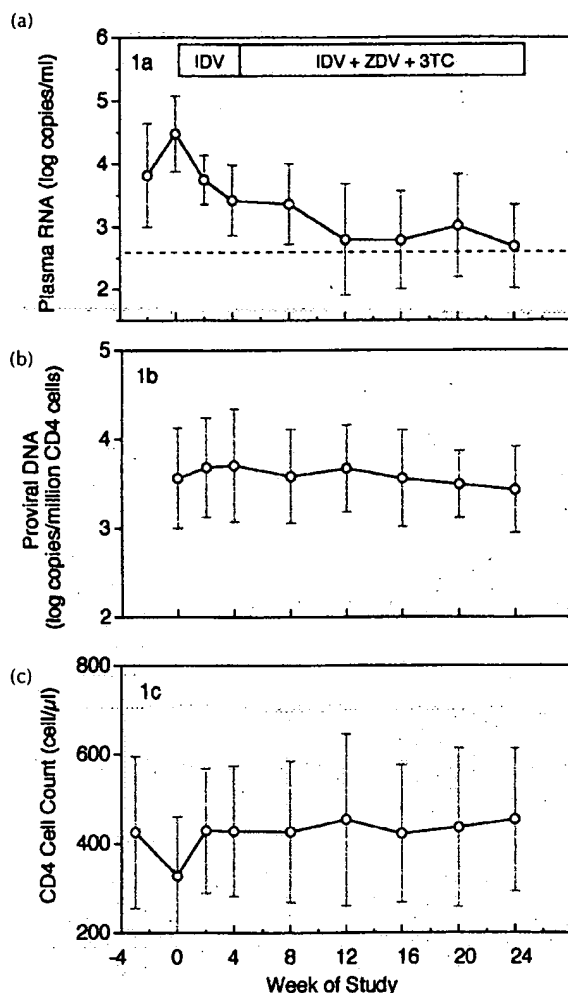


Fig. 1. (a) Changes in plasma HIV RNA, (b) PBMC proviral DNA, and (c) CD4+ T-cell counts throughout the study. Weeks -4 to 0, washout; weeks 0-4, IDV monotherapy; weeks 4-24, ZDV-3TC-IDV combination therapy.

RNA viral load was below 400 copies/ml ($2.6 \log_{10}$ copies/ml) in six out of nine patients at week 24.

PBMC proviral DNA

PBMC proviral DNA levels showed only a minimal drop from $3.56 \pm 0.56 \log_{10}$ DNA copies/ 10^6 CD4+ T cells at baseline to $3.43 \pm 0.48 \log_{10}$ DNA copies/ 10^6 CD4+ T cells at week 24 (Fig. 1b).

CD4+ T-cell counts

CD4+ T-cell counts showed a gradual rise to $453 \pm 160 \times 10^6/l$ by week 24 (Fig. 1c). A wide variation was observed in CD4+ T-cell response of the individual patients.

Mutations

The development of mutations in the HIV protease gene from plasma samples over time is shown for each patient in Fig. 2. Two patients developed the $L_{90}M$

mutation, $G_{48}V$ was found in four, and $V_{82}A$ in three patients. Individual HIV RNA responses and development of resistance mutations varied greatly between patients. We did not find a clear correlation between the mutational patterns that had emerged from previous SQV therapy (and were present at the start of IDV therapy) and the degree of response to IDV therapy, alone or in combination. Neither χ^2 tests, evaluating good and poor responders versus the presence and absence of protease gene mutations, nor t-tests, comparing the mean HIV RNA changes between patients with and without protease gene mutations, showed significant differences between the groups. Furthermore, a lack of correlation between baseline RT mutations and response was also seen, where good and poor responders were equally divided amongst the five patients with a 3TC mutation (M184V), one with an ZDV mutation (K70R), and four with no RT resistance mutations (data not shown).

Discussion

We studied the clinical utility of IDV in SQV-experienced patients and the development genotypic changes in the protease gene resulting from the initial SQV and subsequent IDV therapy. We attempted to identify correlates between genotypic and clinical cross-resistance patterns. Although the design of the study allowed for only 4 weeks of IDV monotherapy, as more data became available our growing concerns regarding a washout and monotherapy phase led us to discontinue enrollment after 10 patients, limiting our ability to define clear correlates between clinical and genotypic resistance. The need to continually address ethical considerations in light of emerging data has become an important part of the practice of clinical trials with antiretroviral agents [25,26].

Patients showed a $1.2 \log_{10}$ copies/ml reduction in plasma HIV RNA at week 4 on IDV monotherapy, continuing to $1.94 \log_{10}$ copies/ml below baseline at week 24 with the addition of ZDV and 3TC. An increase of $0.58 \log_{10}$ copies/ml had been seen during the washout phase; therefore, a more conservative clinically relevant assessment would be a reduction of $1.36 \log_{10}$ copies/ml at week 24. Although a longer period of IDV monotherapy would have allowed a clearer quantification of the efficacy of IDV in this patient population, when designing the study we did not feel further protease inhibitor monotherapy to be an ethical option even in a small group of patients. In addition, the initial month of monotherapy allowed us to clearly demonstrate that IDV remained a potent agent in some of these patients, while the addition of ZDV and 3TC for the remainder of the study more accurately reflected the current clinical use of IDV. The study design there-

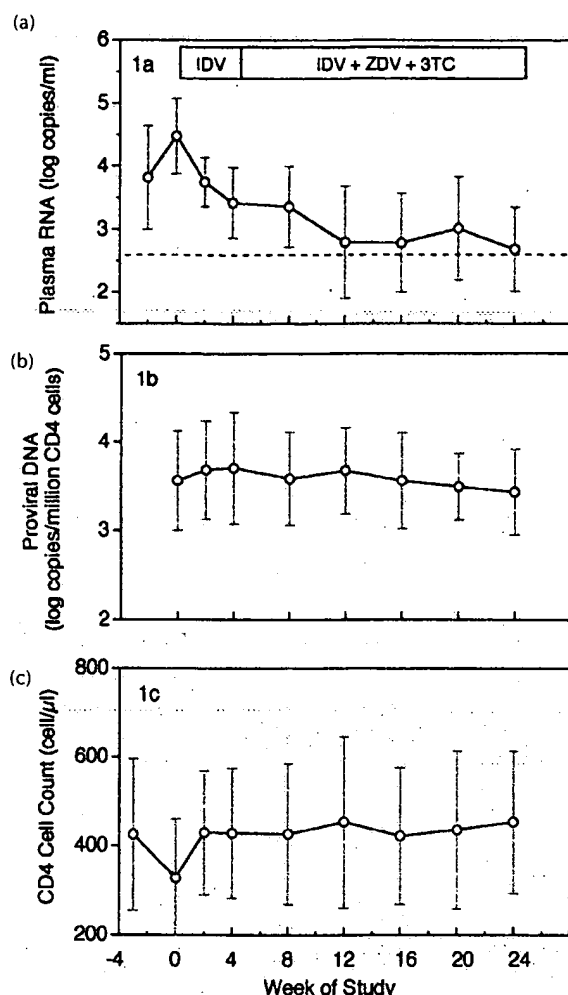


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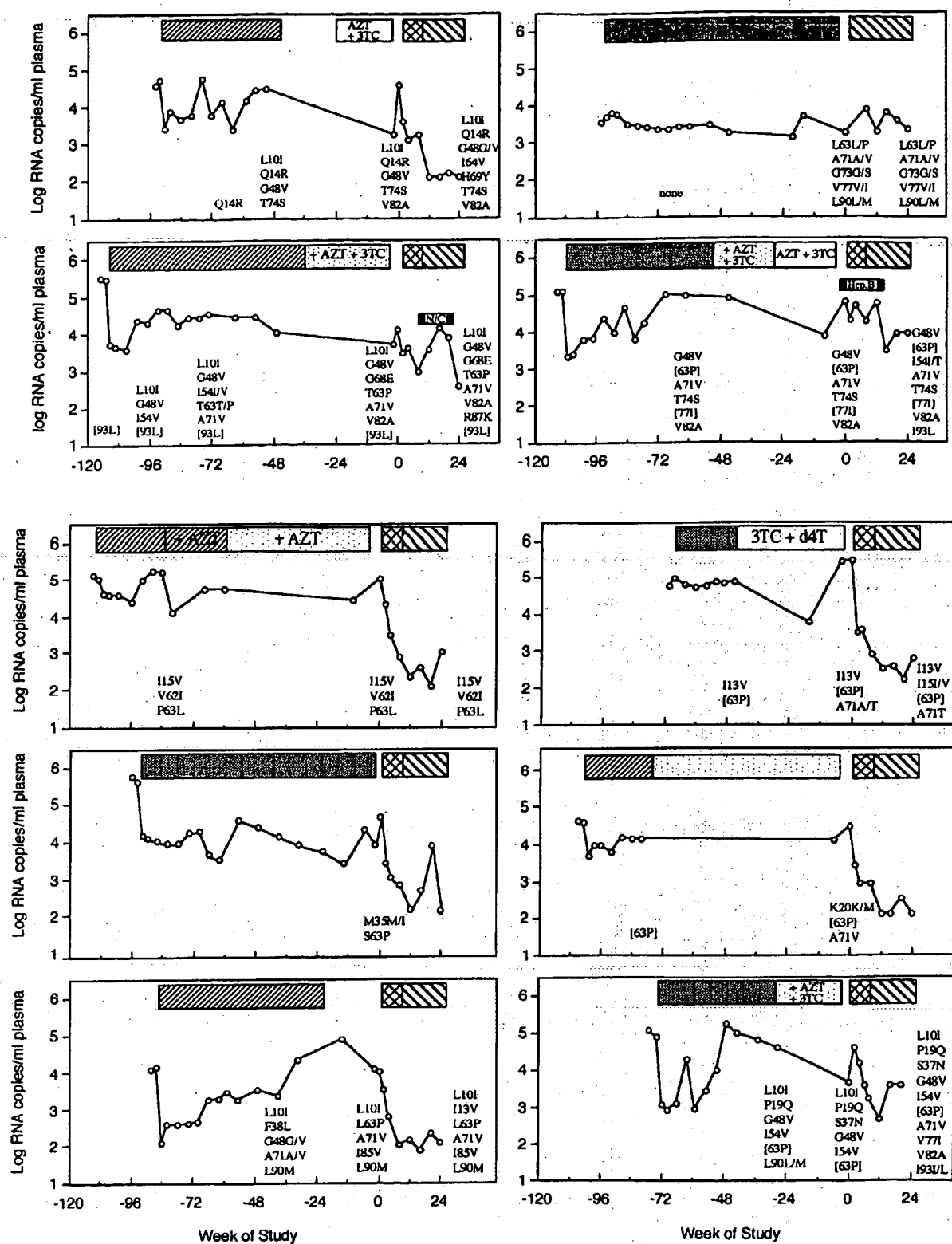


Fig. 2. Genotypic changes in the HIV-1 protease gene determined at different timepoints during and following initial therapy with SQV and subsequent therapy with IDV. Data shown for each individual patient superimposed on changes in plasma HIV RNA and timing of drugs administered.

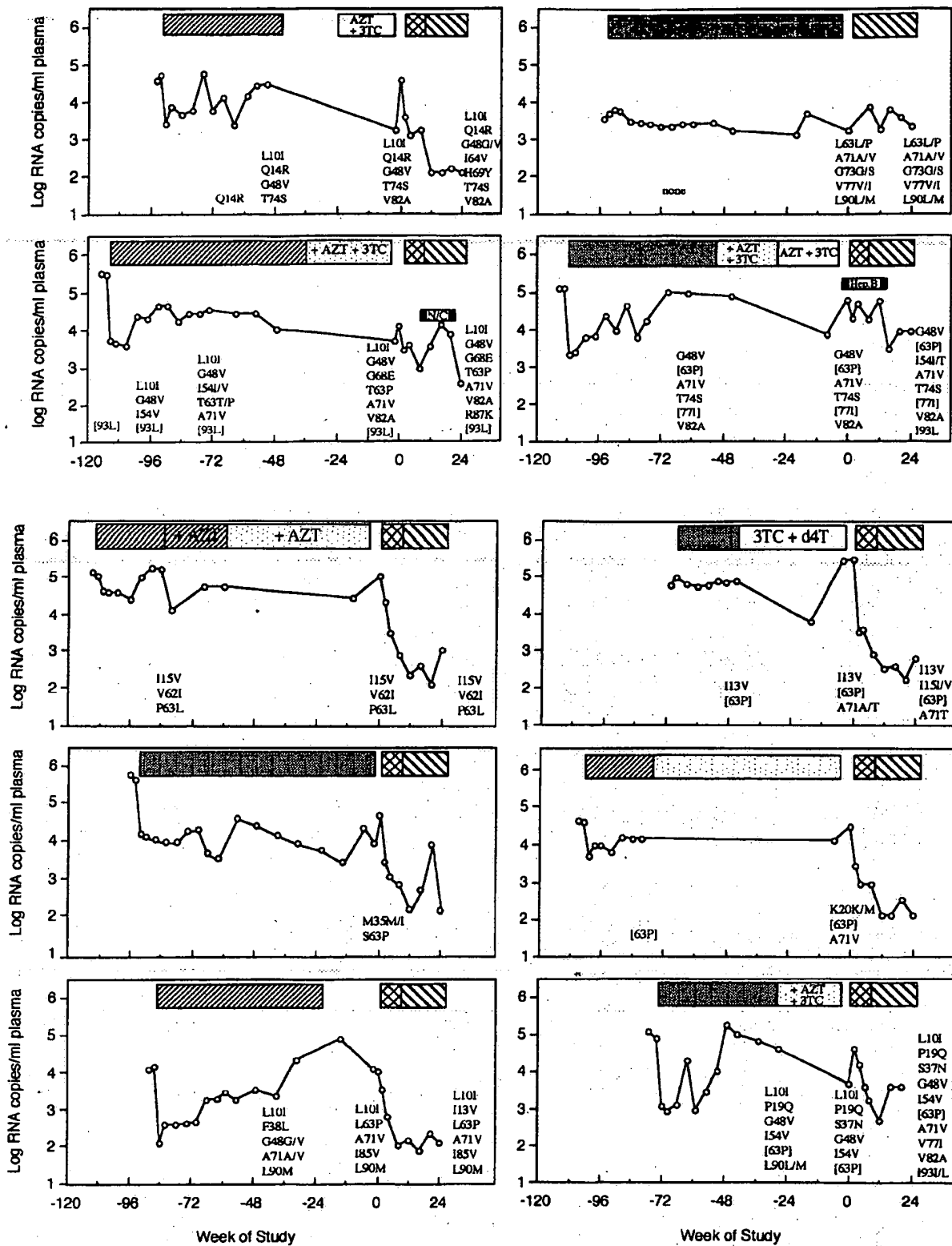


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fore did not allow us to separate the effect of IDV from that of the RTI drugs beyond week 4, but provided data for the combination commonly used in clinical practice. The reduction in HIV RNA viral load appears to be inferior to that reported with protease inhibitor-naïve patients receiving ZDV-3TC-IDV, although a reduction of greater than $2 \log_{10}$ copies/ml was seen in some patients at week 24 [7,8]. This would suggest that IDV remains a potent inhibitor of HIV replication in some SQV-experienced patients. The inability of short-term protease inhibitor therapy to greatly reduce PBMC proviral DNA levels was consistent with our previous findings [4].

Prolonged therapy with high-dose SQV (58 weeks), the majority of which was given as a single drug, resulted in multiple mutations in the protease gene. Six of the 10 patients developed five or more mutations. In three of the four patients in whom the G48V developed, it was accompanied by L10I. The rate of G48V and its association with L10I are greater than those reported with standard dose SQV therapy [11]. Relatively higher rates of G48V were reported in the early *in vitro* studies of SQV and were suggested by our previous study of high-dose SQV [4,27]. This may be due to the greater exposure to the drug found both in culture and when higher blood levels are achieved. The appearance of the V82A mutation has been reported in the past as a result of standard dose SQV therapy but at a far lower rate than seen here [11]. This relatively high rate of mutations following SQV therapy may be due to the fact that the drug was given as monotherapy for prolonged periods of time, at sufficiently high blood levels to apply selective pressure and often following the loss of viral load suppression. This would serve to confirm the concept that administering protease inhibitors as monotherapy, with continued use following the loss of viral load suppression, increases the chance of developing multiple resistance mutations in the protease gene. This is also supported by our finding that additional mutations developed in the protease gene in patients in whom durable viral load suppression was not subsequently achieved with the combination of ZDV-3TC-IDV. In one patient, four additional mutations had developed in the 24 weeks of combination therapy. In a larger dataset including samples from additional studies, we found *in vitro* cross-resistance to IDV in heavily SQV-pretreated patients who had developed multiple mutations [28]. Recently, retrospective analyses in protease inhibitor-experienced patients have correlated genotypic and phenotypic resistance with clinical failure of subsequent protease inhibitor-containing regimens [29-31].

This study provides evidence that continued use of SQV monotherapy at potent doses in the presence of elevated HIV RNA viral load levels results in the development of multiple resistance mutations, which

may lead to early failure of a second protease inhibitor [12,13]. This would support current recommendations of monitoring HIV RNA levels and switching therapy early when levels increase and discouraging the use of protease inhibitors as monotherapy [32]. Our findings that individual resistance patterns and clinical responses varied greatly between patients, together with recent retrospective correlations between specific resistance patterns with clinical failure, suggests that resistance assays may be useful in identifying which patients will benefit from salvage therapy with a second protease inhibitor [33]. Large-scale prospective studies will hopefully help define these assays' role as a clinical tool.

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Activity of a ritonavir plus saquinavir-containing regimen in patients with virologic evidence of indinavir or ritonavir failure

Steven G. Deeks*, Robert M. Grant*[†], George W. Beatty*, Christopher Horton[†], Jill Detmer[‡] and Scott Eastman[§]

Objective: To evaluate the virologic activity of a ritonavir plus saquinavir-containing regimen in patients who have failed an indinavir or ritonavir-containing regimen.

Design: Patients were identified through a retrospective study evaluating the incidence of indinavir or ritonavir failure in our clinic.

Patients: Eighteen patients failing indinavir or ritonavir therapy and who switched to a ritonavir-saquinavir-containing regimen were evaluated. Indinavir or ritonavir failure was defined as a plasma viral load >1500 copies/ml (branched DNA) after 16 weeks of continuous therapy.

Interventions: All patients switched to ritonavir (400 mg twice daily) plus saquinavir (400 mg twice daily) and received concurrent therapy with two nucleoside reverse transcriptase inhibitors (NRTI). Twelve of the 18 patients modified their NRTI regimen at the time ritonavir-saquinavir was initiated.

Outcome measures: Plasma viral load was monitored using a branched DNA assay. Genotypic analysis was performed using a point mutation differential hybridization technique, and was confirmed with direct sequencing.

Results: Fourteen out of 18 patients completed at least 24 weeks of therapy; the remaining four patients discontinued therapy after week 12 due to a lack of virologic response or intolerance. Plasma viral load decreased a median 1.4 log₁₀ after 4 weeks of treatment with ritonavir-saquinavir. Only four patients had a greater than 0.5 log₁₀ decrease in viral load after 24 weeks of therapy. In eight out of 10 patients evaluated, the V82A mutation was present at the time of the switch to ritonavir-saquinavir. Viral rebound on ritonavir-saquinavir was associated with the emergence of mutations at amino acids 46, 48, 54 and 90.

Conclusion: The combination of ritonavir, saquinavir and two NRTI resulted in a moderate but transient suppression of viral replication in patients who have failed indinavir or ritonavir therapy. Failure of ritonavir-saquinavir may be associated with the emergence of mutations associated with resistance to ritonavir/saquinavir monotherapy, particularly the L90M mutation.

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Keywords: Antiretroviral therapy, protease inhibitors, resistance

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Note: Presented in part at the 37th International Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Toronto, Canada, October 1997.

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Requests for reprints to: Dr Steven G. Deeks, Assistant Clinical Professor of Medicine, UCSF, 995 Potrero Avenue, San Francisco General Hospital, San Francisco CA 94110, USA.

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Introduction

Protease inhibitors have had a dramatic impact on the management and natural history of HIV disease [1]. In minimally pretreated patients, indinavir (IDV), when used in combination with zidovudine and lamivudine, results in durable suppression of viral replication [2]. Recent data, however, indicate that a significant number of patients eventually exhibit evidence of virologic failure to IDV or zalcitabine (RZV) therapy [3–5]. Advanced immunodeficiency, prior nucleoside reverse transcriptase inhibitor (NRTI) therapy and non-adherence all contribute to protease inhibitor drug failure [3–7].

Virologic failure in the presence of IDV or RZV is typically associated with predictable mutation patterns [8]. *In vitro*, these mutations confer high-level phenotypic cross-resistance to other protease inhibitors, including nelfinavir and saquinavir (SQV). Although the significance of these mutations in predicting response to subsequent protease inhibitor therapy *in vivo* remains unknown, switching from IDV or RZV to a second protease inhibitor is unlikely to result in durable viral suppression [6,7]. Current US clinical guidelines recommend considering dual protease inhibitor containing regimens for patients who have failed an IDV- or RZV-containing regimen [6,7].

Due to favorable pharmacokinetic interactions, the combination of RZV and SQV, used at reduced doses, has undergone clinical evaluation [9–11]. In protease inhibitor-naïve patients with moderately advanced HIV disease, RZV plus SQV results in durable viral suppression [10]. The efficacy of this combination in protease inhibitor-experienced patients is unknown. Therefore, we analyzed patients who failed an IDV- or RZV-containing regimen and who switched to a RZV-SQV-containing regimen. Genotypic resistance patterns, both before and after the switch, were analyzed in a subset of patients.

Methods

Study population

All patients were followed at the University of California, San Francisco AIDS program, based at San Francisco General Hospital (SFGH). This urban, university-based public hospital clinic provides comprehensive primary care to HIV-infected adults. The majority of patients (99%) are covered by public assistance programs or are uninsured. In 1996, the majority of patients in the clinic were men (86%) and Caucasian (56%).

Patients were identified through a retrospective cohort study evaluating the incidence of virologic failure of

IDV- or RZV-containing regimens in our clinic. Using a database that monitors outpatient visits by primary health-care provider, we identified the names of all patients seen on a regular basis (at least three times) by the same clinician between March 1996 and September 1997. Medical records for each patient were reviewed to identify those who received IDV or RZV for at least 16 weeks, and who had virologic evidence of drug failure. In establishing this cohort of IDV or RZV failures, drug failure was defined as having two consecutive viral load determinations above 500 copies/ml after week 16 of treatment (branched DNA assay; sensitivity, 500 copies/ml).

Inclusion/exclusion criteria

From this cohort, we identified all patients who switched to a RZV-SQV-containing regimen after failing IDV or RZV therapy. Only patients with two consecutive viral load determinations above 1500 copies/ml prior to the switch to RZV-SQV were included in this study. This viral load cut-off was chosen because the specificity of the branched DNA assay (Quantiplex HIV-1 bDNA version 2.0; Chiron, Emeryville, California, USA) at this level is greater than 99%. At the assay's quantification limit of 500 copies/ml, the specificity is approximately 97% (D. Chernoff, personal communication, 1998).

All patients switched from a regimen containing two NRTI and IDV or RZV to a regimen containing two NRTI and RZV (400 mg twice daily) plus SQV (400 mg twice daily). Patients with prior SQV or nelfinavir experience were excluded from this analysis. Patients who received a non-NRTI at or after the switch were also excluded. Patients remained in this analysis only if they had at least 24 weeks of follow-up or stopped therapy prior to week 24 due to intolerance or lack of virologic response.

Data collection

Baseline data, including prior antiretroviral exposure, were obtained at the time IDV or RZV was initially prescribed, and at the time of the switch to a RZV-SQV-containing regimen. Laboratory tests (plasma HIV-1 RNA and absolute CD4+ T-cell levels) were performed in a central facility based at SFGH. Plasma viral load assays were performed using a branched DNA assay (Chiron; lower level of detection, 500 copies/ml).

Genotypic analysis

Between September 1996 and September 1997, a subgroup of patients in this study had been enrolled in a prospective study evaluating the predictive role of genotypic testing when switching protease inhibitor therapy. Plasma was obtained prior to the switch and during subsequent follow-up visits and stored at -70°C. Patients and clinicians did not receive the results of genotypic analysis prior to switching therapy.

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Due to favorable pharmacokinetic interactions, the combination of RTV and SQV, used at reduced doses, has undergone clinical evaluation [9–11]. In protease inhibitor-naïve patients with moderately advanced HIV disease, RTV plus SQV results in durable viral suppression [10]. The efficacy of this combination in protease inhibitor-experienced patients is unknown. Therefore, we analyzed patients who failed an IDV- or RTV-containing regimen and who switched to a RTV-SQV-containing regimen. Genotypic resistance patterns, both before and after the switch, were analyzed in a subset of patients.

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Genotypic analysis was performed using two methods. Point mutations of interest (D30N, M46I/L, G48V, I54V, V82A, I84V, L90M) were analyzed using a differential probe hybridization assay (Chiron) [12]. After PCR amplification, codons of interest were assayed individually using specific pairs of probes that preferentially bind to either the mutant or wild-type sequence. Controls for hybridizations with mutant probes consisted of a biotinylated synthetic oligonucleotide containing both mutant probe and a probe for quantifying the total amount of target sequences. HIV-1_{HXB-2} was used as the wild-type control. Direct sequencing of the entire protease gene was performed on PCR product using an automatic sequencer (Applied Biosystems, Foster City, California, USA). All sequences were proofread manually and aligned with HIV-1_{SF2} as a consensus sequence.

Results

Patients

A total of 741 HIV-positive patients were seen at least three times by 10 SFGH AIDS Program clinicians between March 1996 and September 1997. After a review of their medical records, 129 patients were identified who had virologic evidence of IDV or RTV failure. From this group, a total of 18 patients met our inclusion/exclusion criteria and were included in this study. An additional 11 patients were identified who failed IDV or RTV and switched to RTV-SQV. These patients were excluded due to having a viral load less than 1500 copies/ml at the time of the switch ($n = 1$), prior SQV therapy ($n = 3$), prior nelfinavir therapy ($n = 1$), use of nevirapine after switching to RTV-SQV ($n = 3$), and limited follow-up when this analysis was performed ($n = 3$).

Baseline characteristics prior to initiating IDV or RTV and at the time of the switch to a RTV-SQV-containing regimen are summarized in Table 1. One patient was on RTV at the time of the switch, whereas the rest were receiving IDV. All 18 patients received concurrent therapy with two NRTI at the time of the switch to RTV-SQV. At the time of the switch, 12 patients initiated at least one new NRTI to which they had not been previously exposed. There was a median delay of 2.3 months between the diagnosis of IDV or RTV failure and the switch to RTV-SQV; only five patients switched within 1 month of IDV or RTV failure.

Of the 18 eligible patients, 14 received at least 24 weeks of continuous therapy with RTV-SQV. Four patients discontinued therapy between week 12 and 16, either due to a lack of virologic response ($n = 3$) or due to intolerance ($n = 1$). The latter patient had a limited

Table 1. Baseline characteristics.

| Characteristic | Median values |
|---|---------------|
| Total patients studied (n) | 18 |
| Men | 17 |
| Prior ritonavir | 4 |
| Pre-protease inhibitor baseline | |
| CD4 cells ($\times 10^6/l$) | 73 |
| Viral load (\log_{10} copies/ml) | 4.93 |
| Duration of indinavir or ritonavir therapy (months) | |
| Prior to switch | 8.3 |
| After exhibiting evidence of failure and prior to switch* | 2.3 |
| Baseline values at the time of the switch | |
| CD4 cells ($\times 10^6/l$) | 172 |
| Viral load (\log_{10} copies/ml) | 4.41 |
| Prior nucleoside analogue therapy (months) | 59 |
| Modified nucleoside analogues (n) | 12 |
| Duration of therapy with ritonavir-saquinavir (months) | 10.9 |

*The date of indinavir or ritonavir failure was assumed to be the date the plasma viral load first became detectable (for patients who had an undetectable viral load) or the week-16 date (for patients who never achieved an undetectable viral load).

virologic response at the time RTV-SQV was discontinued.

Virologic outcomes

The plasma viral load response to RTV-SQV is illustrated in Fig. 1. At week 4, plasma viral load decreased a median 1.43 \log_{10} copies/ml below baseline (range, 0.17 to $-2.4 \log_{10}$ copies/ml; $P = 0.005$, week 4 compared with baseline, Wilcoxon matched pairs test). By week 12, viral load had returned towards baseline (median, 0.41 \log_{10} copies/ml change below baseline; range, $+0.39$ to $-2.4 \log_{10}$ copies/ml).

Of the 18 patients studied, three had evidence of a durable response to RTV-SQV. One patient's viral load became undetectable and remained so for 9 months before subsequently rebounding towards baseline. Two patients had a durable 1 \log_{10} decrease in viral load (through week 24). All three patients modified their NRTI regimen at the time of the switch.

The duration of response (defined as a greater than 0.5 \log_{10} decline in viral load below baseline) was a 140 days (median) for subjects who modified their NRTI combination at the time of the switch ($n = 12$), and 63 days (median) for those who did not ($n = 6$; $P = 0.03$, Wilcoxon matched pairs test).

Genotypic resistance

Genotypic analysis was performed using a differential hybridization technique [12] and, when possible, by automated sequencing. Amino-acid differences relative to HIV-1_{SF2} as a consensus wild-type sequence are presented in Fig. 2. Baseline samples were available for 10 patients. Samples were obtained at the day of the switch to RTV-SQV ($n = 7$) or within 60 days of the switch ($n = 3$). All baseline samples studied had mutations consistent with IDV resistance (Fig. 2). The

Genotypic analysis was performed using two methods. Point mutations of interest (D30N, M46I/L, G48V, I54V, V82A, I84V, L90M) were analyzed using a differential probe hybridization assay (Chiron) [12]. After PCR amplification, codons of interest were assayed individually using specific pairs of probes that preferentially bind to either the mutant or wild-type sequence. Controls for hybridizations with mutant probes consisted of a biotinylated synthetic oligonucleotide containing both mutant probe and a probe for quantifying the total amount of target sequences. HIV-1_{HXB-2} was used as the wild-type control. Direct sequencing of the entire protease gene was performed on PCR product using an automatic sequencer (Applied Biosystems, Foster City, California, USA). All sequences were proofread manually and aligned with HIV-1_{SF2} as a consensus sequence.

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The duration of response (defined as a greater than 0.5 \log_{10} decline in viral load below baseline) was a 140 days (median) for subjects who modified their NRTI combination at the time of the switch ($n = 12$), and 63 days (median) for those who did not ($n = 6$; $P = 0.03$, Wilcoxon matched pairs test).

Genotypic resistance

Genotypic analysis was performed using a differential hybridization technique [12] and, when possible, by automated sequencing. Amino-acid differences relative to HIV-1_{SF2} as a consensus wild-type sequence are presented in Fig. 2. Baseline samples were available for 10 patients. Samples were obtained at the day of the switch to RTV-SQV ($n = 7$) or within 60 days of the switch ($n = 3$). All baseline samples studied had mutations consistent with IDV resistance (Fig. 2). The

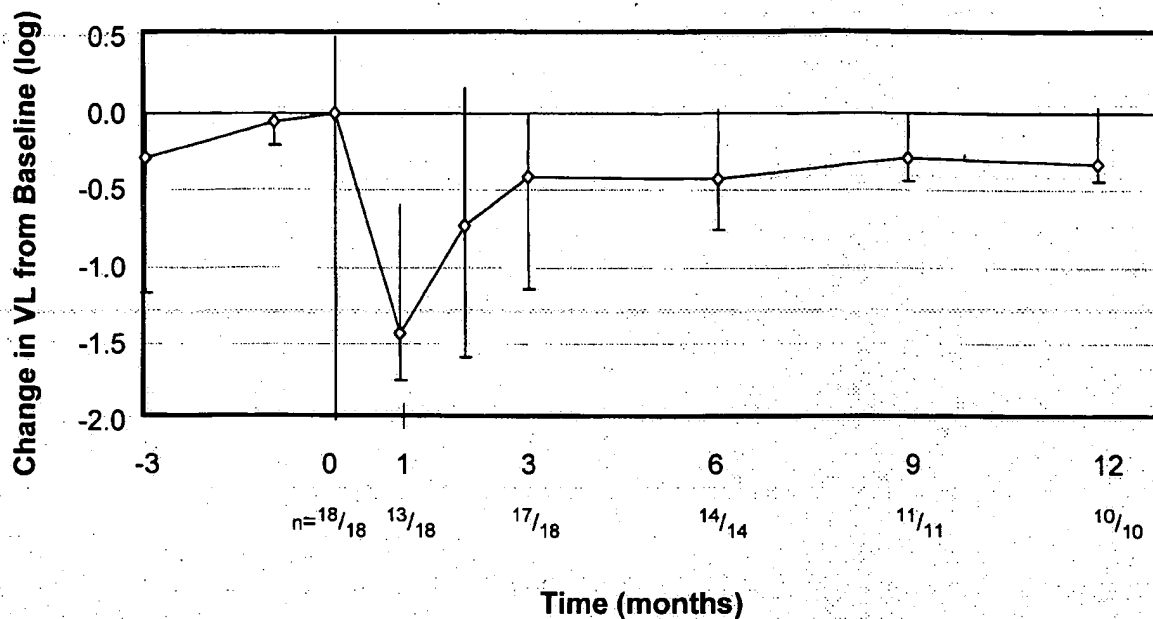


Fig. 1. Change in viral load (VL; median log₁₀ RNA copies/ml) over time after switching from an indinavir- or ritonavir (RTV)-containing regimen to a RTV-saquinavir (SQV)-containing regimen; 25–75% interquartile range is shown. The number of patients with an evaluable viral load at each timepoint over the number of patients still on a RTV-SQV-containing regimen is indicated (n).

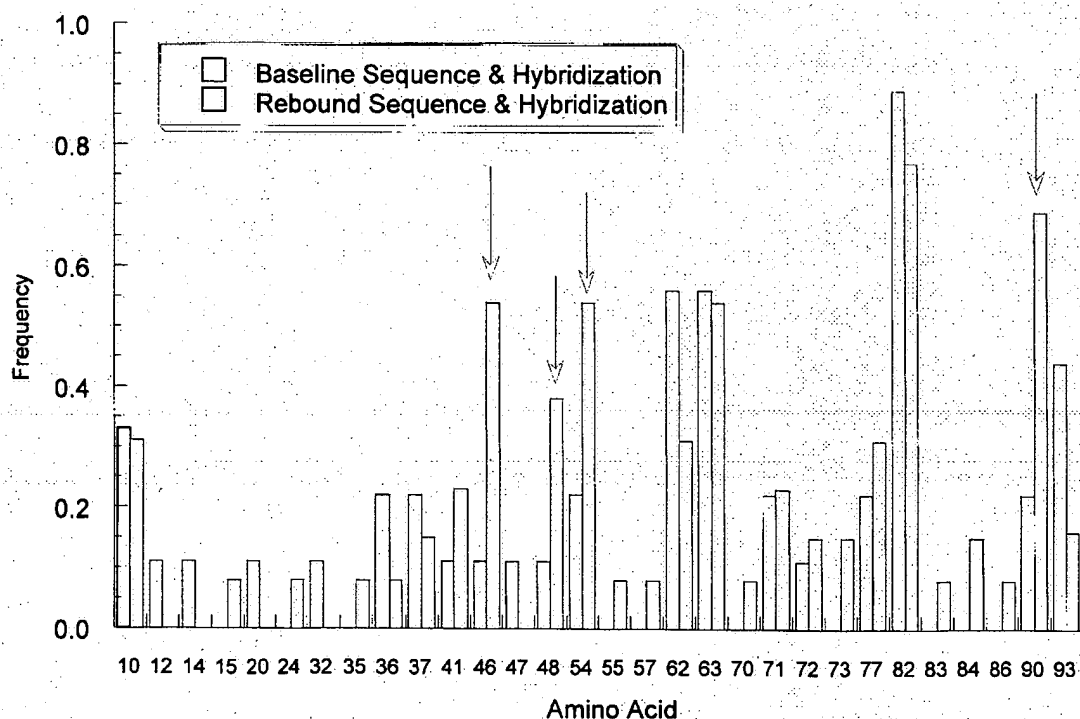


Fig. 2. Mutational patterns observed at the time of initiation and failure of ritonavir-saquinavir therapy. The distribution of amino-acid changes from HIV-1_{SF2} are presented. Hybridization results were available for all samples analyzed. When possible, all coding changes as determined by sequence analysis are presented (six out of 10 at baseline and seven out of 13 at failure). When sequencing results were not available, hybridization results are presented. Sequencing and hybridization results were concordant within the limits of sensitivity of the sequencing reactions.

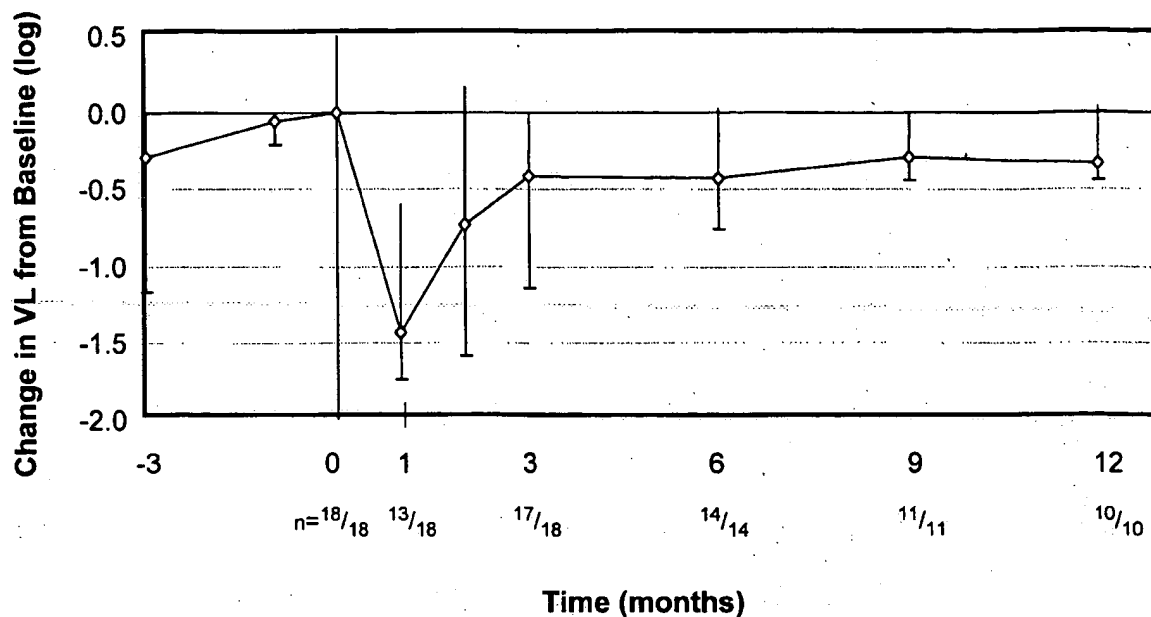


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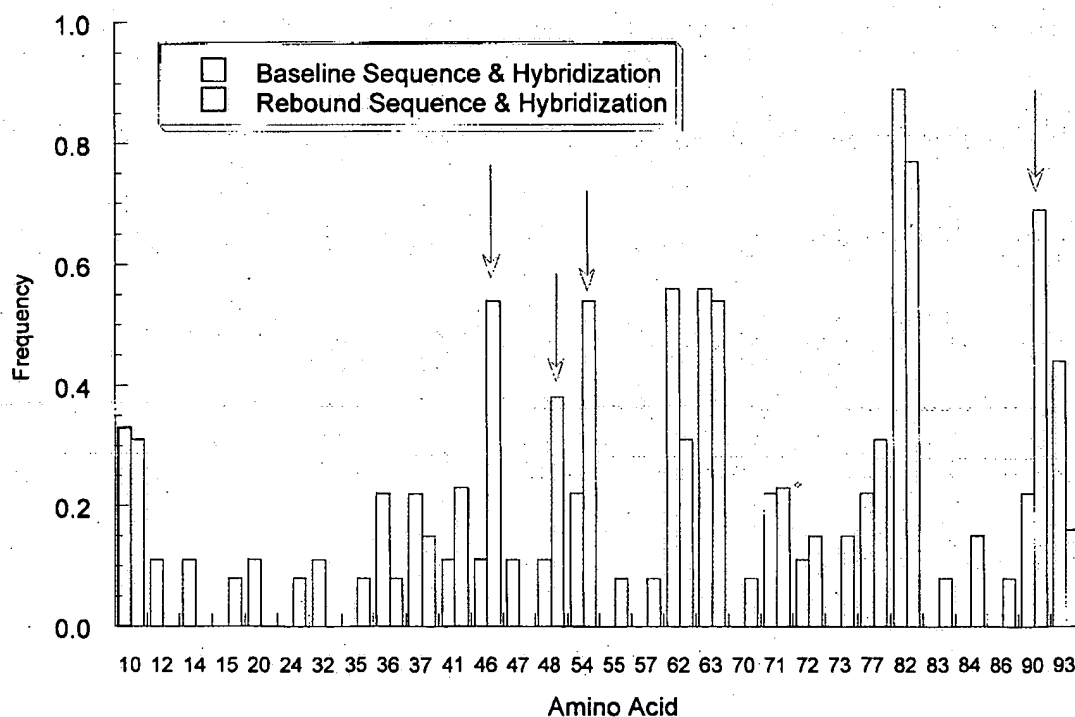


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V82A mutation was present in eight out of 10 patients. A G48V mutation, commonly observed with SQV resistance, was present at baseline in one patient failing IDV despite no prior history of SQV use.

Follow-up samples were available for 13 patients. Resistance patterns associated with virologic failure of RTV-SQV are presented in Fig. 2. Nine of 13 patients failing RTV-SQV had or developed an L90M mutation. Although an increase in the frequency of mutations was also observed at codons 46, 48, and 54, with changes at codons 48 and 54 often appearing together, the increase in frequency associated with a rebound in viral load was greatest at codon 90.

As discussed above, three patients had a potent response to RTV-SQV for at least 24 weeks: one patient had an undetectable viral load through week 24 and two patients had a 1 log₁₀ decrease in viral load through week 24. Notably, none of the three patients had an L90M mutation at baseline. For the two patients with sufficient plasma virus at week 24 to analyze, neither had developed the L90M mutation while on RTV-SQV.

Discussion

Antiretroviral treatment failure is now generally defined on the basis of plasma viral load [6,7]. Although no standard definition for antiretroviral failure exists, clinical guidelines recommend that the aim of therapy is to decrease plasma viral load to levels below the limit of quantification of currently available assays (400–500 copies/ml). Patients who fail to achieve this goal may consider switching to a subsequent antiretroviral regimen, modifying all drugs if possible [6,7]. The decision to switch therapy should be weighed against the concern that there are a limited number of antiretroviral agents, and that failure of the new regimen may limit future options [6]. Although specific recommendations are not available, dual protease inhibitor therapy with RTV and SQV, with concurrent NRTI, has been suggested as one possible salvage regimen after IDV or RTV failure [6]. In this study, we report that the combination of RTV and SQV, with two NRTI, had a moderate but transient effect on plasma viral load in the majority of patients who had failed an IDV- or RTV-containing regimen.

Controversy persists surrounding the issue of protease inhibitor cross-resistance. *In vivo*, IDV and RTV select for overlapping resistance mutations [8,13]. Phenotypic cross-resistance between these two drugs is common. The mutation patterns selected by nelfinavir and SQV are distinct, suggesting that sequential therapy with

these protease inhibitors may be possible. In this study, the use of RTV-SQV (which results in high serum concentrations of SQV) did not result in durable viral suppression after IDV or RTV failure. The use of other dual protease inhibitor-containing regimens, including nelfinavir plus SQV, requires additional studies.

Several factors may have contributed to the lack of response to RTV-SQV. Medical adherence to therapy is an important determinant of a successful outcome with combination therapy [6,7]. Due to the retrospective nature of this study, adherence was not measured. The delay in switching to RTV-SQV may also be an important factor. By delaying the switch, ongoing viral replication in the presence of IDV or RTV occurs. With time, additional mutations may emerge. Theoretically, switching therapy early may prevent the emergence of high-level drug resistance and possibly cross-resistance, thus allowing a more durable response [14]. In this study, of the five patients who switched within 1 month of failing IDV or RTV, none achieved a durable 1 log₁₀ decrease in viral load or became undetectable through 24 weeks of treatment. Determining the importance of switching early will require larger studies. Finally, clinical guidelines strongly recommend changing all antiretroviral therapies when modifying therapy due to drug failure. In this study, initiating at least one new NRTI at the time of the switch had a modest benefit. Presumably, results for the entire group would have been improved had all patients changed to a completely new antiretroviral regimen.

Dual protease inhibitor regimens are undergoing clinical evaluation. In protease inhibitor-naïve patients, the combination of RTV plus SQV had a durable and potent effect on viral load. Approximately 90% of patients achieved evidence of durable viral suppression (<500 copies/ml for 48 weeks) [10]. Viral rebound was associated with the emergence of V82A and I54V in the limited number of patients studied; I84V and M36I were also seen. L90M and G48V, both typically seen with SQV failure, were not observed [15].

In contrast, both G48V and L90M were common in this study of patients failing RTV-SQV therapy. In those patients with timepoints available for analysis, loss of viral suppression on RTV-SQV was temporally associated with L90M. Since virtually all patients failing IDV therapy possessed virus that would be expected to be genotypically resistant to RTV (i.e., the presence of V82A was common), the emergence of SQV-related mutations is not surprising. The presence of L90M may therefore result in cross-resistance to both RTV and SQV and an abbreviated response to the salvage therapy with these drugs. Three patients in this study had a durable (greater than 24 weeks) and potent response to RTV-SQV. In each case, the absence of an L90M mutation at baseline and during follow-up was

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remarkable. The potential of the L90M mutation as a marker of broad protease inhibitor cross-resistance requires further *in vitro* and *in vivo* investigation.

As a retrospective single-center study, this analysis has limitations. Patients were treated by their primary care physician and not followed in a prospective study. Therefore, the use of concurrent NRTI and the timing of viral load determinations were not standardized. Due to the retrospective nature of this study, a selection bias may have been introduced. Any bias, however, was minimized by including all patients who were identified through a large retrospective cohort study, including those patients who discontinued therapy early.

In summary, our data indicate that RTV plus SQV, with concurrent NRTI therapy, has a moderate but transient anti-HIV effect in the majority of patients failing IDV or RTV-containing regimens. Modifying NRTI at the time of the switch provided modest additional benefit. Although virologic failure on RTV-SQV was associated with a wide variety of genotypic mutations, L90M was common. Achieving durable viral suppression after IDV or RTV failure is difficult with a RTV-SQV-containing regimen. More aggressive therapies, such as regimens containing non-NRTI or experimental agents, may be necessary.

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Human Immunodeficiency Virus Type 1 Protease Genotypes and In Vitro Protease Inhibitor Susceptibilities of Isolates from Individuals Who Were Switched to Other Protease Inhibitors after Long-Term Saquinavir Treatment

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An understanding of the mechanisms of virologic cross-resistance between human immunodeficiency virus type 1 protease inhibitors is important for the establishment of effective treatment strategies for patients who no longer respond to their initial protease inhibitor. Protease gene sequencing results from patients treated with saquinavir showed significant increases in the frequency of the G48V protease mutation in patients receiving higher doses of the drug. In addition, all six patients who developed the G48V mutation during saquinavir therapy developed the V82A mutation either on continued saquinavir or after a switch to nelfinavir or indinavir. In vitro susceptibility assays showed that all 13 isolates with reduced susceptibilities to two or more protease inhibitors had either the G48V or L90M mutation, along with an average of six other protease mutations. Reduced susceptibility to nelfinavir was found in 14 isolates, but only 1 possessed the D30N mutation. These results suggest that mutations selected in vivo by initial saquinavir therapy may provide more cross-resistance to the other protease inhibitors than has been previously reported.

Protease inhibitors have become a very important class of drugs for the treatment of human immunodeficiency virus infection (reviewed in references 5 and 13). Significant decreases in plasma virus levels and elevations in CD4 T-cell counts are typically found in patients receiving any of the four currently approved protease inhibitors. This effect is relatively short-lived, however, in patients receiving protease inhibitors as monotherapy, and triple-drug therapy with combinations of protease and reverse transcriptase inhibitors is now recommended.

Mutations in the protease gene have been shown to be involved in conferring reduced susceptibilities of virus isolates to protease inhibitors. Most of these associations have been confirmed by in vitro experiments, and many have been associated with clinical failure in patients and the reduced susceptibilities of isolates from these patients (3, 15, 17). In vitro selection experiments have indicated that several mutations that appear in the protease gene seem to be associated only with certain protease inhibitors. Although some unique mutational patterns appear in vivo when protease inhibitors are administered as monotherapy in protease inhibitor-naïve patients, several mutations appear to be associated with all protease inhibitors. For example, the protease mutation L90M has been reported to appear most frequently in saquinavir-treated patients (8) but also is found after treatment with other protease inhibitors (4, 15, 17). In contrast, the G48V mutation, which appears less frequently than L90M, appears to be associated almost exclusively with saquinavir therapy.

In vitro cross-resistance among proteases appears to range from minor to complete, depending on the particular mutation or combination of mutations studied. Little data, however, has been published regarding clinical cross-resistance to protease

inhibitors, i.e., information regarding the outcome for patients who fail to maintain viral load suppression on their first protease inhibitor and are switched to another. As clinical use of protease inhibitors increases, a greater number of patients who no longer respond to their initial drug regimens will be seen. Options for a subsequent treatment regimen are currently ill defined.

In this study, protease gene mutations in plasma virus and in vitro susceptibility data from viral isolates from patients who failed to maintain viral load suppression on saquinavir therapy and were subsequently switched to nelfinavir or indinavir were examined. Specimens were obtained from patients involved in three clinical studies at Stanford whose clinical results were presented elsewhere, as follows. (i) In a study of antiretroviral-drug-naïve patients who received either 3,600 or 7,200 mg of saquinavir monotherapy per day for 6 months (18), patients who showed benefits from the drug (as defined by elevated CD4 cell counts from baseline and/or suppression of viral load to below baseline) were allowed to continue saquinavir treatment and to receive reverse transcriptase inhibitors. (ii) A subset of patients from the first study were entered into a follow-up study in which they were switched to indinavir monotherapy for 4 weeks and then received zidovudine and 2',3'-dideoxy-3'-thiacytidine for 20 additional weeks (19). (iii) A third study involved 16 saquinavir (1,800 mg/day)- and reverse transcriptase inhibitor-experienced patients whose protease inhibitor was switched to nelfinavir (12).

Protease gene sequences from the plasma of patients were determined by reverse transcription and PCR methods previously described (21), except that the reverse transcription and first-round PCR primers were MAW-26 (TTG GAA ATG TGG AAA GGA AGG AC) and RT21 (16). Second-round PCR primers were PRO1 (19) and RT20 (16). Sequencing of the second-round product was performed by using dye-labelled dideoxy-terminator kits (Applied Biosystems, Foster City, Calif.) with primers proseq (AAG AGA GCT TCA GGT TTG G) and PSR2 (ATG CCT TTA TTT TTT CTT CTG TC).

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Protease gene sequences from the plasma of patients were determined by reverse transcription and PCR methods previously described (21), except that the reverse transcription and first-round PCR primers were MAW-26 (TTG GAA ATG TGG AAA GGA AGG AC) and RT21 (16). Second-round PCR primers were PRO1 (19) and RT20 (16). Sequencing of the second-round product was performed by using dye-labelled dideoxy-terminator kits (Applied Biosystems, Foster City, Calif.) with primers proseq (AAG AGA GCT TCA GGT TTG G) and PSR2 (ATG CCT TTA TTT TTT CTT CTG TC).

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TABLE 1. Frequencies of protease mutations in saquinavir-treated patients

| Mutation | % of patients with mutation at saquinavir dose (mg/day) of: | |
|--------------|---|-----------------------------|
| | 1,800 ^a | 3,600 or 7,200 ^b |
| L10I or L10V | 40 | 41 |
| M46I | 0 | 0 |
| G48V | 5 | 35 ^c |
| L63P | 73 | 70 |
| A71V | 56 | 47 |
| V82A | 5 | 18 |
| I84V | 3 | 6 |
| L90M | 40 | 41 |

^a Data from reference 8; 37 to 55 patients treated for a mean of 46 weeks (range, 32 to 60 weeks).

^b 17 patients treated for a mean of 77 weeks (range, 40 to 124 weeks).

^c $P = 0.0012$ (by Fisher's exact test).

To rule out laboratory contamination of sequencing results, the uniqueness of each sequence result was confirmed by analyzing nucleotide sequence divergence among all sequences generated in the laboratory. Genotypic mixtures were reported when the minority population was at least 30% of the total.

Reports describing protease mutations in patients treated with 1,800 mg of saquinavir per day have indicated that the L90M mutation occurs with the highest frequency (2, 8). This mutation is also seen in patients treated with all other protease inhibitors (4, 5, 17, 22). The G48V mutation has also been shown to appear with saquinavir therapy, although at a substantially lower frequency than L90M (8), and appears to be relatively unique to saquinavir. In this study, protease gene sequences were obtained from 16 patients who received either 3,600 or 7,200 mg of saquinavir monotherapy per day for 63 ± 28 weeks (mean \pm standard deviation) (range, 24 to 104 weeks) and had an average overall saquinavir exposure (with or without reverse transcriptase inhibitors) of 77 ± 28 weeks (range, 40 to 124 weeks). Results shown in Table 1 indicate that there was a significant increase in the frequency of the G48V mutation in patients who had received the higher doses of saquinavir (3,600 or 7,200 mg/day), compared to published data from similar patients who had received the standard (1,800 mg/day) dose of saquinavir for a mean of 46 weeks (8). There was also an increase in the frequency of the V82A mutation, although this was not statistically significant. The frequencies of other protease inhibitor mutations did not differ between saquinavir doses. Because there was a slight difference in treatment duration between the patients in the low- and high-dose saquinavir studies, it is difficult to completely separate the impact of dose and/or total drug exposure from the impact of duration of treatment. Nevertheless, since higher doses of saquinavir provide greater plasma drug levels and greater viral load suppression (18), these higher doses of saquinavir resulted in greater selective pressure on the virus, which elicited the G48V mutation with greater frequency.

All patients who developed the G48V mutation on saquinavir therapy eventually developed the V82A mutation either on continued saquinavir therapy (three of six patients) or after a switch from saquinavir to nelfinavir (two of six) or indinavir (one of six). Two recent reports have also identified this dual genotype emerging from saquinavir-treated patients (7, 20). For two patients for whom the V82A mutation was not evident after saquinavir therapy by population-based sequencing, analysis of 10 to 12 molecular clones derived from PCR amplicons (TA Cloning Kit; Invitrogen, Carlsbad, Calif.) of plasma virus

RNA showed the presence of small populations ($\leq 20\%$) of V82A-containing viruses. These results indicate that the G48V-V82A genotypes that developed during saquinavir therapy were expanded and potentiated during subsequent therapy with either nelfinavir or indinavir. The emergence or persistence of the G48V-V82A genotype after a change in protease inhibitor therapy is consistent with *in vitro* susceptibility data (Table 2) that shows these isolates to have reduced sensitivities to nelfinavir, saquinavir, and indinavir.

A total of 31 primary viral isolates obtained from 23 patients treated with saquinavir or with saquinavir followed by nelfinavir were evaluated for sensitivities to nelfinavir, saquinavir, and indinavir (Table 2). The 90% inhibitory concentration (IC_{90}) for each isolate was determined by the ACTG/DOD consensus method (1). In addition, the sequences of the viral stocks used for susceptibility testing were determined from viral RNA, as described above for plasma virus. Nucleotide sequences from the viral stocks were compared to the nucleotide sequences of the patients' plasma virus sequences to rule out laboratory contamination of sequencing or susceptibility results. The results showed that seven isolates from five patients had reduced sensitivities to saquinavir, nelfinavir, and indinavir. Four of those isolates were from patients who received only saquinavir therapy, while two were from patients who received nelfinavir after saquinavir. Isolates from six patients had reduced susceptibilities to saquinavir and nelfinavir but maintained sensitivity to indinavir. Four of these isolates were from patients who had received nelfinavir after saquinavir, and two were from patients who had received only saquinavir. Isolates from 17 patients who had failed to maintain viral load suppression on either low- or high-dose saquinavir were sensitive to all three protease inhibitors. There was no significant correlation between duration of saquinavir treatment and reduced sensitivity to any protease inhibitor.

All of the primary patient isolates (Table 2) that had reduced susceptibilities to more than one protease inhibitor possessed either the G48V or L90M mutation, along with an average of 6.4 additional protease gene mutations. The G48V mutation was significantly associated with resistance to two or more protease inhibitors (4 of 13 isolates with resistance versus 0 of 17 isolates without resistance [$P = 0.026$, by Fisher's exact test]). The L90M mutation was not significantly associated with resistance to two or more protease inhibitors, as 6 isolates with the L90M mutation were sensitive to all three protease inhibitors (9 of 13 isolates with resistance versus 6 of 17 isolates without resistance [$P = 0.155$, by Fisher's exact test]). In this and other published studies, the L90M mutation alone has had little measurable impact on susceptibilities to saquinavir (15), nelfinavir (17), ritonavir (15), and indinavir (15). The accumulation of additional protease mutations, more than four in this study, appears to be necessary for significant resistance to protease inhibitors. The identification of the type and nature of the mutations necessary for conferring protease inhibitor resistance will require analysis of a larger number of L90M-containing virus isolates and/or *in vitro* mutagenesis studies.

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TABLE 2. Relationships between protease mutations and in vitro susceptibilities to protease inhibitors in primary patient isolates^a

| Reduced susceptibility to ^b | Patient | IC ₅₀ (μM) ^c | | | Patient protease inhibitor experience (mo) | Amino acid change in viral isolate protease compared to consensus sequence B at position: | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|---------|------------------------------------|------|---------|--|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--|--|--|
| | | SOV | NFV | IDV | | 10 | 14 | 15 | 20 | 30 | 35 | 36 | 37 | 41 | 46 | 48 | 53 | 54 | 57 | 60 | 62 | 63 | 71 | 73 | 74 | 77 | 82 | 84 | 90 | 93 | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SOV, NFV, IDV | 397 | 8.08 | 9.80 | 1.57 | SOV (10) → NFV (4) | I | R | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1299 | 6.00 | 1.91 | 9.14 | SOV (7) → NFV (4) | I | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1306 | 1.18 | 2.08 | 0.66 | SOV (10) → NFV (4) | I | | V | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1306 | 0.92 | 0.65 | 0.57 | SOV (10) | I | | V | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 397 | 0.58 | 1.78 | 1.09 | SOV (10) | | R | V | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 19 | 0.58 | 1.82 | 0.65 | SOV (12) | | E | V | | | | | | | | | | | | | | | | | | | | | | | | | |
| 39 | 0.37 | 0.69 | 0.72 | SOV (6) | | | V | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SOV, NFV | 34 | 0.80 | 0.73 | 0.35 | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1294 | 0.42 | 2.69 | 0.43 | SOV (22) → NFV (4) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1299 | 0.34 | 0.50 | 0.28 | SOV (7) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1304 | 0.21 | 0.91 | 0.41 | SOV (11) → NFV (4) | I | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1296 | 0.20 | 0.64 | 0.32 | SOV (6) → NFV (4) | I | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1295 | 0.20 | 1.13 | 0.22 | SOV (9) → NFV (4) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NFV | 1297 | 0.03 | 0.64 | 0.08 | SOV (11) → NFV (4) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | 28 | 0.09 | 0.12 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 30 | 0.08 | 0.21 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1304 | 0.07 | 0.24 | 0.16 | SOV (11) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1298 | 0.07 | 0.25 | 0.05 | SOV (9) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 21 | 0.07 | 0.13 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1294 | 0.06 | 0.27 | 0.07 | SOV (22) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1295 | 0.06 | 0.23 | 0.15 | SOV (9) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1296 | 0.06 | 0.20 | 0.11 | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1297 | 0.06 | 0.15 | 0.08 | SOV (11) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 13 | 0.06 | 0.09 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 8 | 0.06 | 0.07 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 5 | 0.05 | 0.05 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 6 | 0.04 | 0.01 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1300 | 0.02 | 0.04 | 0.02 | SOV (8) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1 | 0.02 | 0.08 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 0.02 | 0.07 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 18 | 0.01 | 0.05 | NT | SOV (6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None ^d | | 0.03 | 0.07 | 0.12 | Naive | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

^a Abbreviations: SOV, saquinavir; NFV, nelfinavir; IDV, indinavir.^b Reduced susceptibility is defined as an increase in IC₅₀ of at least fourfold over the mean IC₅₀ of 16 primary isolates obtained from 16 protease inhibitor-naïve patients.^c Results are means of two or three tests. NT, not tested.^d Isolates from protease inhibitor-naïve patients (n = 16).

TABLE 2. Relationships between protease mutations and in vitro susceptibilities to protease inhibitors in primary patient isolates^a

| Reduced susceptibility to ^b | Patient | IC ₅₀ (μM) ^c | | | Patient protease inhibitor experience (mo) | Amino acid change in viral isolate protease compared to consensus sequence B at position: | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|---------|------------------------------------|------|--------|--|---|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|--|--|--|
| | | SQV | NFV | IDV | | 10 | 14 | 15 | 20 | 30 | 35 | 36 | 37 | 41 | 46 | 48 | 53 | 54 | 57 | 60 | 62 | 63 | 71 | 73 | 74 | 77 | 82 | 84 | 90 | 93 | | | |
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| | 1306 | 1.18 | 2.08 | 0.66 | SQV(10)→NFV(4) | I | | V | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | 19 | 0.58 | 1.82 | 0.65 | SQV(12) | | E | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SQV, NFV | 39 | 0.37 | 0.69 | 0.72 | SQV(6) | | | V | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 34 | 0.80 | 0.73 | 0.35 | SQV(6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1294 | 0.42 | 2.69 | 0.43 | SQV(22)→NFV(4) | | | | I | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1299 | 0.34 | 0.50 | 0.28 | SQV(7) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1304 | 0.21 | 0.91 | 0.41 | SQV(11)→NFV(4) | | R | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1296 | 0.20 | 0.64 | 0.32 | SQV(6)→NFV(4) | | | | M | | | | | | | | | | | | | | | | | | | | | | | | |
| NFV | 1295 | 0.20 | 1.13 | 0.22 | SQV(9)→NFV(4) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1297 | 0.03 | 0.64 | 0.08 | SQV(11)→NFV(4) | | | V | | N | D | I | | K | | | | | | | | | | | | | | | | | | | |
| | 28 | 0.09 | 0.12 | NT | SQV(6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | 8 | 0.06 | 0.07 | NT | SQV(6) | | E | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| 18 | 0.01 | 0.05 | NT | SQV(6) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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^b Reduced susceptibility is defined as an increase in IC₅₀ of at least fourfold over the mean IC₅₀ of 16 protease inhibitor-naïve patients.

^c Results are means of two or three tests. NT, not tested.

^d Isolates from protease inhibitor-naïve patients (n = 16).

and saquinavir resistance patterns may significantly overlap, similar to what is seen with indinavir and zidovudine resistance patterns (3).

Although one clinical study indicated that nelfinavir did not provide a durable response for a group of highly antiretroviral-drug-experienced patients who had previously taken saquinavir (12), further clinical studies will be needed with different patient populations to ascertain whether nelfinavir, when used with a more potent combination regimen, can be an effective treatment option for patients who fail to maintain viral load suppression on saquinavir. While published data regarding the genotypes of patients who fail to durably respond to saquinavir and switch to indinavir has been lacking, recent reports suggest that patients who possess the L90M mutation and switch to indinavir (6, 19) maintain the L90M mutation and add additional mutations, similar to what has been reported after a switch to nelfinavir (12).

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Genotypic Changes in Human Immunodeficiency Virus Type 1 Associated with Loss of Suppression of Plasma Viral RNA Levels in Subjects Treated with Ritonavir (Norvir) Monotherapy

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Ten subjects received 600 to 1,200 mg of the human immunodeficiency virus type 1 (HIV-1) protease inhibitor ritonavir per day. Following 2 weeks of therapy, plasma HIV RNA levels decreased by a mean of 1.57 (range, 0.89 to 1.96) log units. With continued therapy, HIV RNA levels began to rise in eight subjects. The initial rise in plasma RNA levels was temporally associated with the development and quantitative increase in the V82 resistance mutation. Doubling times of the V82A mutant virus were estimated to be 2.4 to 4.8 days. An L63P/A mutation was commonly present at baseline even in subjects with a durable virologic response. The concomitant acquisition of an L63P/A mutation with the V82A/F mutation at the time when plasma RNA levels rebounded suggests a role for the L63P/A mutation in improving the fitness of the V82A/F mutation. Subsequent additional genotypic changes at codons 54 and 84 were often associated with further increases in plasma RNA levels. Ongoing viral replication in the presence of drugs resulted in the appearance of additional genotypic changes, including the L90M saquinavir resistance mutation, and decreased phenotypic susceptibility. The relative fitness of the protease V82A ritonavir resistance mutation and reverse transcriptase T215Y/F zidovudine resistance mutation following drug withdrawal were estimated to be 96 to 98% that of the wild type. Durability of the virologic response was associated with plasma RNA levels at the nadir. A virologic response beyond 60 days was not observed unless plasma HIV RNA levels were suppressed below 2,000 copies/ml, consistent with estimates from V82A doubling times for selection of a single resistance mutation to dominate the replicating population.

Efforts to treat human immunodeficiency virus type 1 (HIV-1)-infected individuals have been directed towards the development of inhibitors of viral enzymes necessary for viral replication. The viral reverse transcriptase (RT) is the target of most drugs now in use. Virus variants with reduced susceptibility to several of these drugs have been selected in vitro (2, 11, 46) and have emerged with therapy in vivo (22, 42, 44). Genotypic changes in the RT gene following long-term zidovudine (ZDV) therapy have been associated with phenotypic losses in sensitivity to ZDV (21, 22). Establishing the in vivo relationship between the development of genotypic resistance and changes in plasma HIV RNA levels during ZDV monotherapy, however, required the development of methodologies to measure plasma HIV RNA levels and to determine the amounts of genotypic variants in a population. Recently correlations of the development of genotypic resistance in plasma virus RNA with the loss of viral suppression in vivo have been demonstrated for the K70R mutation with ZDV (9) and the M184V mutation with lamivudine (42). Interestingly, the K70R mutation is associated with only an eightfold decrease in susceptibility to ZDV in vitro (21) while the M184V mutation results in a >500-fold decrease in susceptibility to lamivudine (46). Similarly, with the nonnucleoside RT inhibitors, such as delavirdine and nevirapine, the Y181C mutation results in

>100-fold reduction in drug susceptibility in vitro (37) and has been associated with loss of HIV suppression in patients (12, 37, 39).

A new class of drugs has been evaluated in phase I/II clinical trials (8, 26). The target of these drugs is the HIV protease which is required for cleavage of the Gag and Gag-Pol polyproteins and production of infectious virus (20, 35). As with the RT inhibitors, HIV variants with reduced susceptibility to protease inhibitors have been isolated in vitro (15, 18, 25, 34). Characterization of these drug-resistant variants has revealed several changes in the protease amino acid sequence which have been associated with low-level (<10-fold) decreases in phenotypic susceptibility to a number of inhibitors (6, 7, 28). Some of these mutations result in a reduced susceptibility to more than one protease inhibitor (6, 7, 33, 41, 47).

We have previously described a differential hybridization assay for determination of relative amounts of wild-type (WT) virus and resistant mutants (MUT) in the plasma HIV RNA of infected individuals (10). The assay is linear and reproducible over a wide dynamic range. Here we apply this technology to detect increases in the amount of plasma virus with specific mutations in association with increases in plasma HIV RNA levels following treatment with ritonavir (Norvir). Molla et al. (28) have described an ordered accumulation of mutations during ritonavir therapy based on the frequency of mutations observed. Here we describe an ordered pattern of accumulation based on the accumulation of mutations associated with changes in viral load during ritonavir therapy. Furthermore,

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TABLE 1. Description of hybridization probes

| Probe | Sequence | Location ^a (nt) | Temp (°C) | |
|--------------|---------------------|-------------------------------|---------------|------|
| | | | Hybridization | Wash |
| M46 WT | ACCAAAAAATGATAGKRGG | 1934–1951 | 45 | 45 |
| M46I MUT | ACCAAAAAATHATAGKRGG | 1934–1951 | 45 | 45 |
| M46L MUT | ACCAAAAATGATAGKRGG | 1934–1951 | 45 | 45 |
| I54 WT | AGGTWTTATYAAAAGTAA | 1958–1974 | 35 | 35 |
| I54V MUT | AGGTWTTGTAAAAGTAA | 1958–1974 | 35 | 35 |
| L63 WT | TCAGATACTYRTAGAAA | 1985–2001 | 45 | 40 |
| L63P MUT | TCAGATACCYRTAGAAA | 1985–2001 | 45 | 40 |
| L63A MUT | TCARATAGCYRTAGAAA | 1985–2001 | 45 | 40 |
| A71 WT | ACATAAAGCTRTAGGTA | 2009–2025 | 45 | 35 |
| A71V MUT | ACATAAAGTTRTAGGTA | 2009–2025 | 45 | 35 |
| V77 WT | GTATTAGTAGGRCCTA | 2028–2043 | 35 | 35 |
| V77I MUT | GTATTAATAGGRCCTA | 2028–2043 | 35 | 35 |
| V82 WT | ACMCCTGTCAACRTAAT | 2042–2057 | 50 | 35 |
| V82A MUT | TACMCCTGCCAACRTA | 2042–2057 | 50 | 35 |
| V82F MUT | TACMCCTTTCAACRTA | 2042–2057 | 35 | 35 |
| I84 WT | TDYCAACATAATTGG | 2048–2062 | 40 | 30 |
| I84V MUT | TDYCAACGTAATTGG | 2048–2062 | 40 | 30 |
| L90 WT | AGRAAYCTGTTRACTCA | 2064–2080 | 45 | 40 |
| L90M MUT | AGRAAYCTGATGACTCA | 2064–2080 | 45 | 40 |
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^a HIV-1_{SF2} (40).

differential hybridization allowed estimates of the doubling times of MUT populations during viral load rebounds and of the fitness of the expanding viral populations. Linear regression analysis of the rate of reversion of MUT populations following cessation of therapy provided an estimate of fitness of the replicating MUT population. These data indicate that the doubling time of the expanding resistant population is short, that the fitness of the resistant population is very close to that of the WT virus, and, that as a result, reversion of MUT populations in the absence of selective pressure is a slow process. Finally, the durability of the virologic response was correlated with the maximum suppression of viral replication, suggesting that the appearance of virus containing multiple mutations is a result of continued selection during ongoing viral replication.

(This study was presented in part at the 3rd Conference on Retroviruses and Related Infections, January 28 to February 1, 1996.)

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Patients and sample material. The subjects represent a subset of the study subjects previously described (26). All subjects on previous antiretroviral therapy discontinued all medications except *Pneumocystis carinii* pneumonia prophylaxis for 2 weeks before therapy was initiated.

HIV-1 RNA quantification. HIV-1 RNA in plasma was quantified by branched-DNA (bDNA) signal amplification-based hybridization with a Quantiplex HIV RNA assay kit (32). Plasma samples with values below the detection limit of the standard assay (10,000 HIV-1 RNA equivalents per ml, expressed as copies per milliliter) were tested by a modified bDNA assay with a detection limit of 500 HIV copies/ml (19).

RT-PCR of the HIV-1 protease gene. Plasma virus RNA was prepared as previously described (16). PCR was performed as a modification of the procedure described by Eastman et al. (10). cDNA synthesis was performed on 10 µl of prepared RNA (100-µl equivalent of the starting plasma). The 100-µl reaction mixture included a buffer that contained 50 mM Tris (pH 8.3), 2.5 mM MgCl₂,

10 mM KCl, 0.1 mg of bovine serum albumin/ml, 0.25 mM (each) deoxynucleoside triphosphate, and 30 pmol each of the primers PROT2083.UP (5'-GGAAG GACACCAAMTGAAAGA-3', bp 1596 to 1616, HIV-1_{SF-2} [40]) and biotinylated PROT2662.DOWN (5'-ATTCCTGGCTTTAACTTTACTGG-3', bp 2127 to 2149). Samples were heated to 65°C for 90 s, cooled to 42°C for 1 min before 200 U of Moloney murine leukemia virus RT (GIBCO/BRL) was added, and incubated at 42°C for 15 min. The samples were heated to 100°C for 2 min, then cooled to 55°C for 5 min, during which time 5 U of *Taq* polymerase (Perkin-Elmer, Norwalk, Conn.) was added. The temperature was raised to 72°C for 3 min, with 40 subsequent cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 3 min to generate a 554-bp fragment.

Analysis of PCR product. For differential hybridization analysis, 10 µl of the PCR product was diluted in 90 µl of phosphate buffer, pH 5.5. Fifty microliters was dispensed to duplicate wells in which streptavidin (XENOPORE Corporation, Saddle Brook, N.J.) was covalently bound and incubated at 50°C for 30 min. The wells were washed, and the bound PCR product was denatured in 0.15 N NaOH for 5 min followed by washing. To the appropriate wells, 100 µl of hybridization solution (5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 1% sodium dodecyl sulfate, 0.5% polyvinylpyrrolidone, 0.5% bovine serum albumin) containing either WT or MUT alkaline phosphatase-labeled probe (Table 1) was added and incubated for 1 h at the appropriate temperature. All wells were washed three times for 5 min each time at the appropriate temperature with prewarmed 1× SSC–0.1% sodium dodecyl sulfate, followed by three washes with prewarmed 1× SSC–0.1% Triton X-100, followed by four room-temperature washes in 1× SSC. For chemiluminescent detection, 50 µl of chemiluminescent substrate (LumiphosTM 530; Lumigen, Detroit, Mich.) was added to each well, incubated at 37°C for 30 min, and measured on a luminometer (Chiron, Emeryville, Calif.). In order to account for the amount of PCR product bound to each well, each well was stripped with 0.15 N NaOH and rehybridized with an alkaline phosphatase-labeled probe to a highly conserved region, the generic (GNR) probe, and washed as described above. The probes, probe sequences, map locations, hybridization temperatures, and wash temperatures are presented in Table 1. Controls for hybridizations with MUT probes were generated by site-directed mutagenesis (25). Alternatively, a biotinylated synthetic oligonucleotide containing both MUT probe and GNR probe target sequences was used as a hybridization control. HIV-1_{HXB2} was used as the WT control.

Estimation of MUT populations. The relative proportions of MUT and WT populations for each probe set was initially determined. The relative light units (RLUs) for each sample hybridized with the WT- or MUT-specific probe were

TABLE 1. Description of hybridization probes

| Probe | Sequence | Location ^a (nt) | Temp (°C) | |
|--------------|--------------------|-------------------------------|---------------|------|
| | | | Hybridization | Wash |
| M46 WT | ACCAAAAATGATAGKRGG | 1934–1951 | 45 | 45 |
| M46I MUT | ACCAAAAATHATAGKRGG | 1934–1951 | 45 | 45 |
| M46L MUT | ACCAAAAYTGATAGKRGG | 1934–1951 | 45 | 45 |
| I54 WT | AGGTWTTATYAAAAGTAA | 1958–1974 | 35 | 35 |
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Method for back calculating viral densities prior to initiation of therapy. The method for back calculating viral densities makes use of CD4⁺ T-cell data to infer the growth rate of MUT viruses at previous time points. The model makes three main assumptions: (i) that viral growth can be described by means of the simple mathematical model presented by Perelson et al. (36), (ii) that peripheral blood CD4⁺ T-cell counts can be taken as a measure of the target cell densities, and (iii) that immune responses that may contribute to the death of infected cells and clearance of free virus do not change appreciably following the initiation of drug therapy. The model of Perelson et al. (36), when applied to MUT virus, consists of the following equations: $dI/dt = \beta VT - \delta I$ and $dV/dt = n\delta I - cV$, where T is the density of target cells, I is the density of infected T cells, V is the density of free HIV, β is the infection rate, δ is the death rate, n is the number of virions released by an infected cell, and c is the clearance rate of free virus. The rate of change of a MUT virus, λ , under these equations can be shown to be

$$\lambda(t) = \frac{-(c + \delta) + \sqrt{(c + \delta)^2 + 4b\delta T}}{2} \quad (1)$$

where $b = n\beta$ is a growth rate parameter characteristic of each virus.

The densities of MUT viruses at previous times were estimated through repeated application of the formula $V(t - \Delta t) = V(t)e^{-\lambda(t)\Delta t}$, where $V(t)$ is the viral density at time t and Δt is the amount of time between successive CD4⁺ T-cell counts. For purposes of back calculation, it was assumed that T is proportional to the density of CD4⁺ T cells (which generally increases as virus declines) but that b , c , and δ are constant. For the values of c and δ , published estimates were used (36). The growth rate parameter b was then estimated by evaluating the solution of equation (1) for b at time points where data existed on both CD4⁺ T-cell counts and the rate of change of MUT virus. In cases where there was more than one estimate for b , the average b was used.

Because the formula $V(t - \Delta t) = V(t)e^{-\lambda(t)\Delta t}$ is only an approximation (this becomes exact as Δt approaches zero), these numerical procedures were tested against simulated data sets in which the number of data points was similar to that in the actual trials. Baseline MUT virus densities estimated by this procedure generally came within about 10% of the true values, indicating that rounding errors in the back-calculation procedure do not seriously distort estimates for the densities of MUT viruses at baseline.

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This method almost certainly underestates the amount of virus at early time points. Previous theoretical treatments, such as those presented by Nowak et al. (30), McLean and Nowak (27), and Stillianakis et al. (45), suggest that the rate of change in the density of MUT virus will increase (often from negative to positive) following the initiation of drug treatment. The simple exponential model, therefore, provides a conservative minimal estimate for the prevalence of MUT virus at the initiation of treatment.

Estimation of fitness of MUT populations following drug withdrawal. The fitness of the MUT population was estimated from the analysis of the replacement of the MUT population with the WT virus in the absence of drugs. This approach assumes replication is continuous in time, where s , the selection coefficient, is the fitness difference between the WT and MUT populations. The selection coefficient can be estimated from a group of data points at various times by linear regression analysis of $\ln[p(t)/q(t)]$, with time as an independent variable (29), where p is the frequency of the more fit WT variant and q is the frequency of the less fit MUT variant. When the lack of detectable WT virus during the follow-up period prevented an estimate of s by the above method, the upper limit

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was estimated to be no less than the mutation rate, 3×10^{-5} ; $p(t)/q(t)$ was estimated as the sensitivity of detection of the differential hybridization assay, 0.01 (10); and t (in days) is the time of follow-up.

Sequence analysis of the protease-coding region of patient plasma RNA. RNA was extracted from plasma virus and PCR amplified as described above. One microliter of this reaction was diluted to 10 μ l and amplified for another 40 cycles with the same primers. Unincorporated primers were removed with a Centricon-100 (Amicon, Inc., Beverly, Mass.), and PCR products were sequenced directly on a DNA sequencer (Applied Biosystems, Foster City, Calif.), with unlabeled GNR probe as a primer. All sequences were proofread manually and aligned with that of HIV-1₅₅₂ as a consensus sequence. Alternatively, a nested PCR strategy was employed, followed by cloning of the PCR product and sequencing.

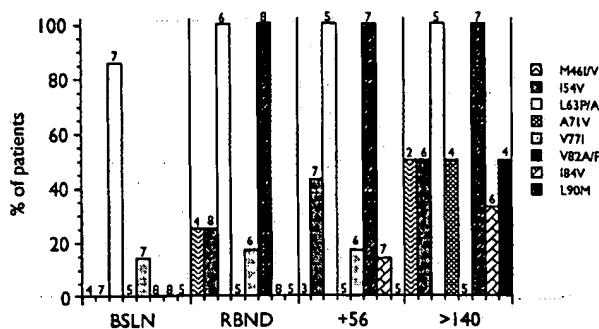


FIG. 1. Time of development of various mutations in the protease gene during ritonavir monotherapy. BSLN, baseline; RBND, viral load rebound; +56, viral load rebound plus 56 days; >140, viral load rebound plus ≥ 140 days. The number of subjects evaluated at each time point is indicated above each bar.

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RESULTS

Identification of resistance mutations associated with rebounds in plasma HIV RNA. The rapid and dramatic antiviral effect of ritonavir on plasma HIV RNA levels has been well documented (8, 14, 26). We studied a subset of these patients (mean CD4⁺-cell concentration, 185/mm³) (14, 26) in whom ritonavir monotherapy produced a mean decrease in plasma HIV RNA levels of 1.7 log units within 2 weeks. A rebound in RNA levels within 1 year of initiation of therapy was observed in 8 of 10 subjects, while 2 of 10 maintained viral suppression. For four subjects who had lost viral suppression, ritonavir dosage was escalated to 1,200 mg daily with little impact on plasma RNA levels.

We analyzed plasma HIV RNA from 10 subjects on ritonavir monotherapy for changes at loci identified as responsible for decreased susceptibility to ritonavir (25, 28). The presence of genotypic changes from the WT consensus sequence at various times for the eight subjects who experienced viral load rebounds are presented in Fig. 1. The bDNA assay has been demonstrated to be able to discern threefold changes in plasma RNA levels as significant (48). For the purposes of this analysis, a threefold (0.5 log₁₀) increase in plasma RNA levels above the nadir on two consecutive determinations was em-

normalized to the respective RLUs hybridized with the GNR probe to account for the quantity of PCR product bound to each well ($WT:GNR_{raw} = WT\ RLUs/GNR_{WT}\ RLUs$; $MUT:GNR_{raw} = MUT\ RLUs/GNR_{MUT}\ RLUs$). Furthermore, these values were normalized to 100% WT and 100% MUT control values to control for the specific activity of the individual probes [$WT:GNR = (WT:GNR)_{test}/(WT:GNR)_{WT\ control}$; $MUT:GNR = (MUT:GNR)_{test}/(MUT:GNR)_{MUT\ control}$]. The portion of MUT species was estimated by multiplication of the plasma HIV RNA levels (HIV copies per milliliter) by the proportion of MUT [HIV copies per milliliter \times $MUT:GNR/(MUT:GNR + WT:GNR)$].

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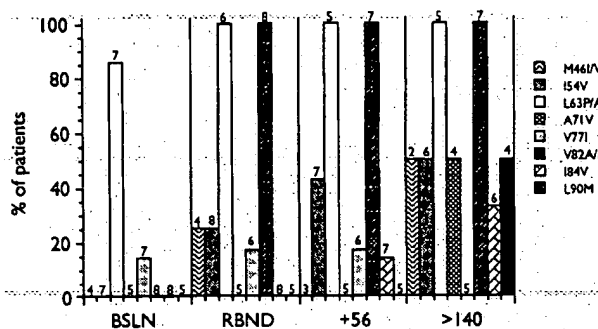


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RESULTS

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We analyzed plasma HIV RNA from 10 subjects on ritonavir monotherapy for changes at loci identified as responsible for decreased susceptibility to ritonavir (25, 28). The presence of genotypic changes from the WT consensus sequence at various times for the eight subjects who experienced viral load rebounds are presented in Fig. 1. The bDNA assay has been demonstrated to be able to discern threefold changes in plasma RNA levels as significant (48). For the purposes of this analysis, a threefold (0.5 \log_{10}) increase in plasma RNA levels above the nadir on two consecutive determinations was em-

ployed to define a viral load rebound. The mean increase in plasma RNA levels over the nadir at the time of viral load rebound was 1.3 (range, 0.66 to 2.53) log units. At the time of the viral load rebound, relatively few mutations were detected, primarily at codons 63 and 82. The majority of subjects, however, had changes at codon 63 at baseline. With continued viral replication in the presence of drug after the viral load rebound, additional genotypic changes are observed.

Alternatively, we analyzed longitudinal samples so that genotypic changes could be temporally associated with changes in plasma HIV RNA. The total HIV RNA copies per milliliter and determinations of various MUT populations by differential hybridization and sequencing for selected subjects are presented in Fig. 2. By differential hybridization, a change at codon 82 (seven of eight, V82A; one of eight, V82F) was temporally associated with the initial loss of viral suppression.

Two patterns of acquisition of the second most common mutation, I54V, representing the I54V-V82A double MUT, were observed. The first was exemplified by subjects 301 and 306, in whom the I54V mutation was acquired well after the appearance of a V82A mutation and viral load rebound and was not associated with a further rise in HIV RNA levels. Both of these subjects also received the lowest dose of ritonavir. In three other subjects receiving higher doses of ritonavir, the I54V mutation (I54V-V82A double MUT) appeared temporally with the V82A mutation; however, the double-MUT population appeared at lower levels and the rate of expansion was slower. In two of the three subjects (304 and 409), complete conversion of the replicating viral population to the double MUT was associated with further increases in viral load, suggesting that the I54V mutation results in a further decrease in susceptibility to ritonavir. The slower expansion of the double-MUT population, however, may indicate reduced fitness.

An L90M mutation was observed in two subjects (301 and 304) only well after the viral load rebound and in the presence of a large number of other mutations. An I84V change was observed in only one subject (310), following the development of the V82A mutation, and was associated with a further increase in viral load.

Three subjects were completely L63P/A at baseline: 306, 313, and 409. Viral RNA was a mixture of L63 WT and L63P MUT at baseline in three subjects, 304, 310, and 401 (Fig. 1), but became completely L63P MUT concomitant with the loss of viral suppression. In four subjects who were mixtures or L63P/A MUT at baseline, initiation of ritonavir therapy coincided with changes to L63 WT, only to return to L63P MUT at the time of the viral load rebound. This is most apparent in the large fluctuations of L63P MUT in subject 310.

As shown in Fig. 2, the phenotypic sensitivity of virus isolated from PBMCs from four subjects (301, 304, 306, and 313) established a progressive decrease in susceptibility to ritonavir with duration of therapy. Sequence analysis of subjects 301 and 304 demonstrated the stepwise accumulation of additional mutations, including K20R, A71V, and L90M. An M46I mutation appeared in subject 304 at day 57 but reverted to M46 WT, while an M36I change was present at baseline and throughout therapy. Similarly, an M46I mutation appeared at the time of rebound in subject 313 by differential hybridization but had reverted to M46 WT 14 days later (data not shown).

In the two subjects who experienced long-term viral suppression below the limit of detection of the bDNA assay, 406 and 408 (Fig. 1), codon 82 remained WT. Furthermore, certain changes from the WT HIV-1_{SF2} consensus sequence present at baseline, some of which have been associated with resistance to protease inhibitors (7, 28), were lost with therapy in these subjects. Most notable were changes at codons 63, 71, and 77.

While this apparent reversion may be affected by the small amounts of RNA introduced into the RT-PCR reactions, the reversion of both subjects 406 and 408, as well as the maintenance of WT sequence in subject 203 (28), argues against this possibility. It is possible, therefore, that this WT virus represents viral replication in a site that is privileged with regard to exposure to ritonavir, such as latently infected cells, brain, or testes.

Kinetic analysis and estimation of fitness from estimates of baseline plasma RNA levels of MUT populations. Four subjects in the lowest-dosage group, 600 mg/day, experienced rebounds in plasma RNA within 42 days of ritonavir monotherapy. At time points when the V82A MUT was detectable above the WT background, it was possible to quantify the rate of expansion of the MUT population. Due to the frequency of time points during the exponential viral load rebound, a fit to the data at these time points on a log scale allowed the determination of the slope of the line as defined by linear regression (minimally, 4 time points) in three of these subjects. From this, an estimate of the doubling time of the V82A MUT virus was determined (Table 2). It is possible that the longer doubling time estimate for subject 312 is a result of longer intervals between time points at the time of the viral load rebound than in the other subjects.

As the steady-state level of the MUT population provides a rough estimate of the selective disadvantage to the viability of the virus, we wished to estimate pretreatment levels of the MUT population. We used a variation of the model of Perelson et al. (36) to estimate the baseline prevalence of the V82A MUT (see Materials and Methods). A maximum estimate of s was calculated by using the hybridization data at the early time points for each subject as the limit of detection of the assay to estimate an upper limit to the amount of V82A MUT, above which the MUT would be detected in the hybridization assay. A conservative minimum estimate of s was calculated from the linear regression analysis described above. Both the CD4⁺ model and the linear extrapolation are presented graphically for these subjects in Fig. 2. The estimated s values are presented in Table 3. The CD4⁺ model estimated fitness for the V82A MUT population at 99% that of the WT population for subjects 306 and 312, with minimum estimates on the order of 94 and 90%, respectively. Subject 301 had estimates of fitness that were much less, to the point that the linear extrapolation produced a value that was less than the mutation rate of HIV (24). Plasma virus from this subject also had no genotypic changes from the consensus WT at the initiation of therapy, as opposed to that from subject 306, which was completely L63P at baseline.

Impact of the presence of RT T215Y/F ZDV resistance mutation on development of the V82A ritonavir resistance mutation. Seven of the 10 subjects' baseline samples demonstrated baseline ZDV genotypic resistance mutations, including the RT T215Y/F mutation. In order to assess the impacts of ritonavir treatment and the development of the V82A protease resistance mutation on the RT T215Y/F locus, selected longitudinal samples from these subjects were analyzed for the amount of T215Y/F MUT. The amount of virus containing the RT T215Y/F mutation for each of the seven subjects, along with the amount of virus containing the V82A mutation, is presented in Fig. 3. The rapid change from V82 WT to V82A MUT in the protease gene in these subjects had no impact on the T215Y/F MUT of the RT. The appearance of T215 WT sequences was observed in four subjects (see below). Only subject 408, a long-term responder, experienced a complete reversion to T215 WT (day 328 of ritonavir therapy).

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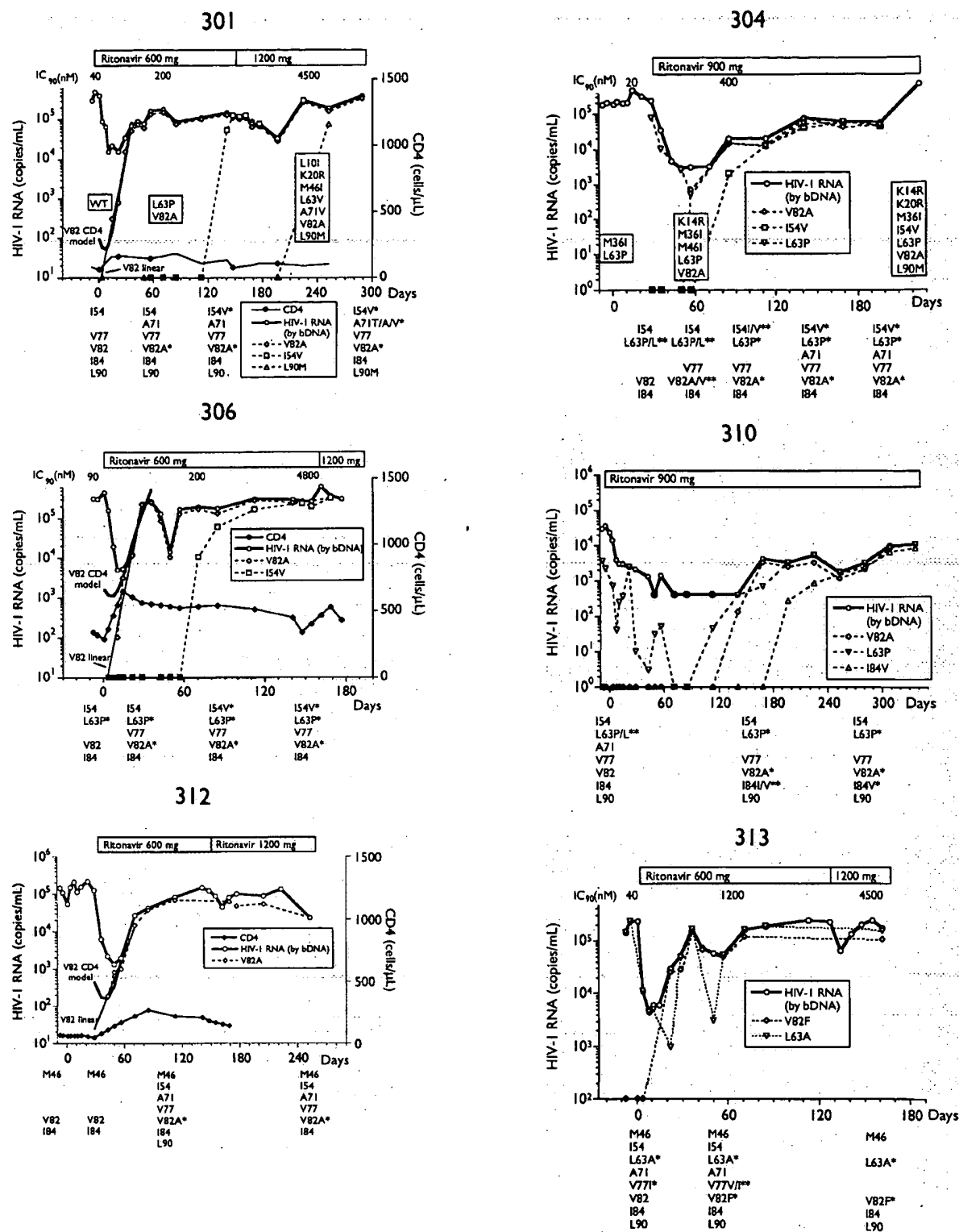
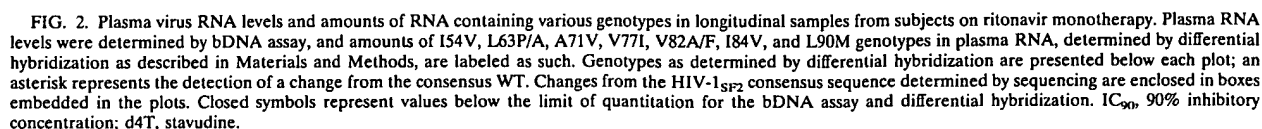


FIG. 2. Plasma virus RNA levels and amounts of RNA containing various genotypes in longitudinal samples from subjects on ritonavir monotherapy. Plasma RNA levels were determined by bDNA assay, and amounts of I54V, L63P/A, A71V, V77I, V82A/F, I84V, and L90M genotypes in plasma RNA, determined by differential hybridization as described in Materials and Methods, are labeled as such. Genotypes as determined by differential hybridization are presented below each plot; an asterisk represents the detection of a change from the consensus WT. Changes from the HIV-1_{SF2} consensus sequence determined by sequencing are enclosed in boxes embedded in the plots. Closed symbols represent values below the limit of quantitation for the bDNA assay and differential hybridization. IC₅₀, 90% inhibitory concentration; d4T, stavudine.



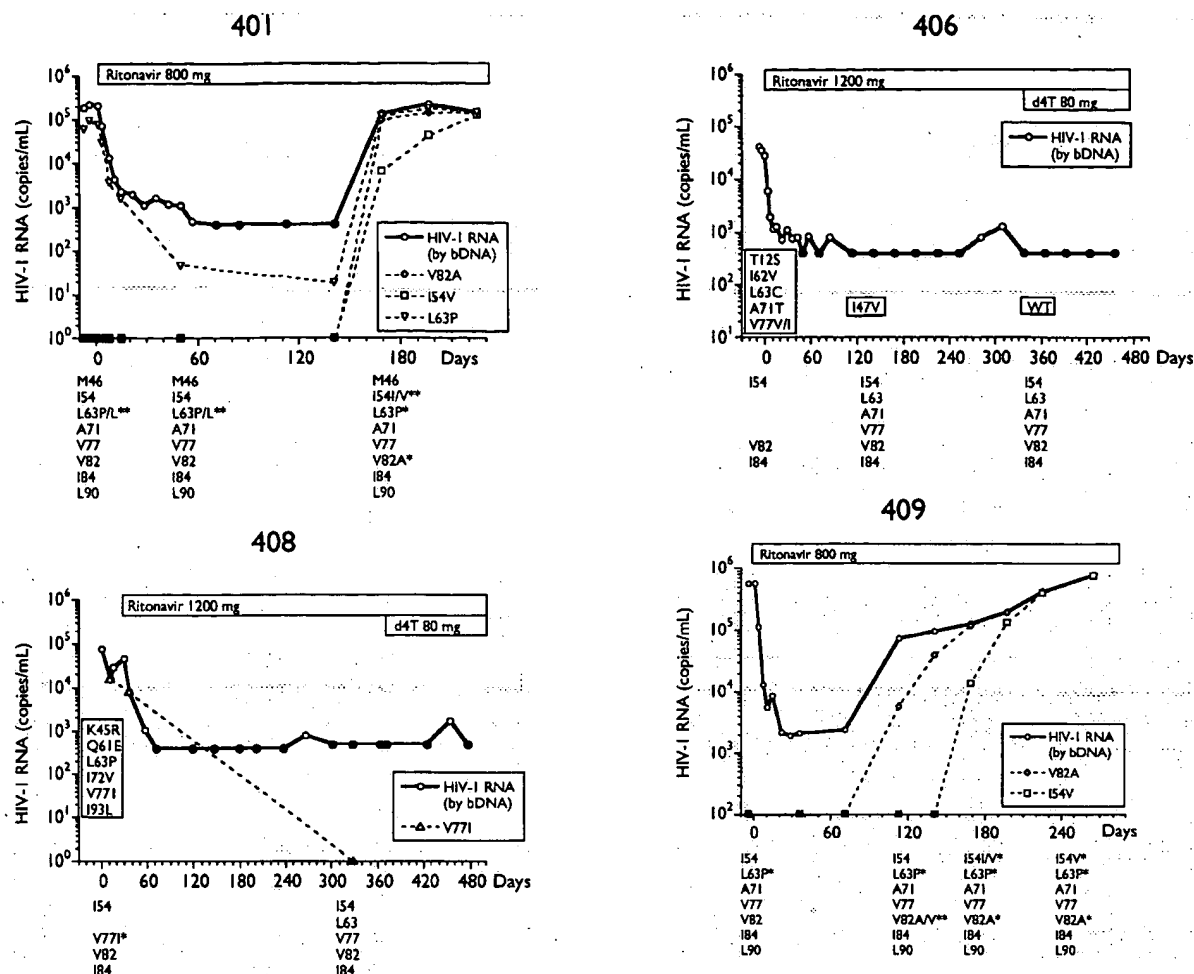


FIG. 2—Continued.

Fitness of MUT virus following drug withdrawal. The relative fitness of a MUT population in the absence of drugs can be calculated by standard formulas to model the effect of replacement of the less fit (MUT) genotype by the more fit (WT) genotype. By knowing the change in the proportion of the MUT and WT populations over time, it was possible to calculate the selective disadvantage (s) of the RT T215Y/F MUT in four subjects in which T215 WT sequences became detectable (Table 4). Only time points at which WT virus was detectable were used for the regression analysis in order to get a more accurate estimate of the rate of change.

In those subjects in which WT sequences were not detectable during the time of follow-up, an upper bound for s was

calculated. To approximate the greatest rate of increase of the WT population, the proportion of the WT population at the end of the study period was taken to be the limit of sensitivity of the differential hybridization assay, 1%. Similarly, the proportion of the WT 1 day into drug withdrawal was conservatively estimated to be the mutation rate of the virus. Limited follow-up data on the stability of the RT T215Y/F from three additional subjects and the V82A MUT from two subjects following withdrawal from ZDV and ritonavir therapy, respectively, was available. Estimates of the upper limit of s were on the order of 0.03, except in subject 409, who had the shortest

TABLE 2. Estimation of doubling times of V82A MUT populations

| Subject | Baseline WT (copies/ml) | Doubling time (days) | 95% Confidence limits | | r^2 |
|---------|-------------------------|----------------------|-----------------------|-------|-------|
| | | | Lower | Upper | |
| 301 | 386,800 | 2.36 | 1.53 | 5.17 | 0.969 |
| 306 | 435,100 | 2.81 | 1.45 | 4.33 | 0.913 |
| 312 | 123,000 | 4.81 | 3.83 | 6.54 | 0.980 |

TABLE 3. Estimation of relative fitness from expansion of the V82A population

| Subject | Baseline RNA (equivalents/ml) | s value | | |
|---------|-------------------------------|-------------------------|------------------------|--------------------------------|
| | | Hybridization (maximum) | CD4 ⁺ model | Linear extrapolation (minimum) |
| 301 | 386,800 | 0.0013 | 0.148 | 4.22 ^a |
| 306 | 435,100 | 0.0002 | 0.010 | 0.059 |
| 312 | 123,000 | 0.0005 | 0.010 | 0.103 |

^a Represents a prevalence less than the rate of mutation.

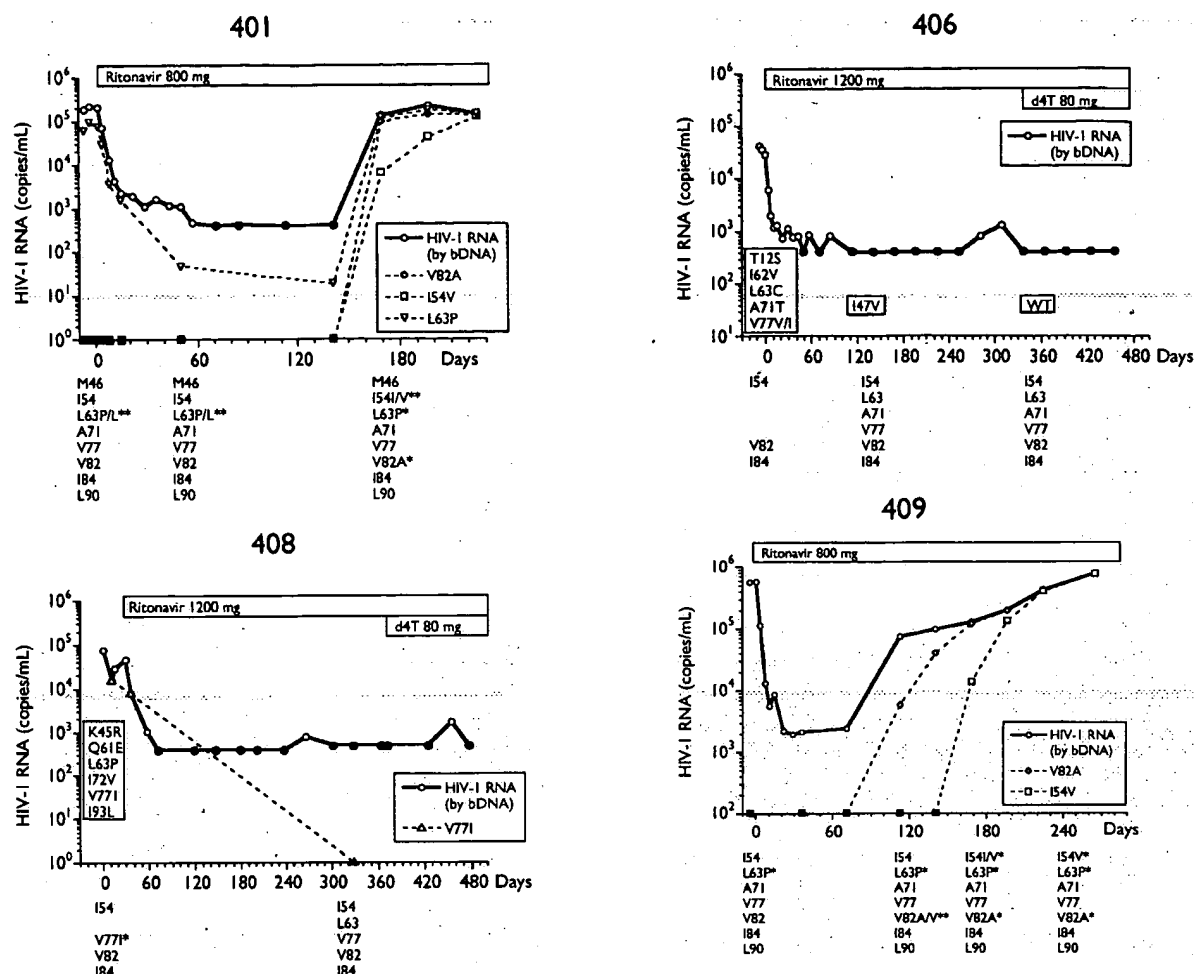


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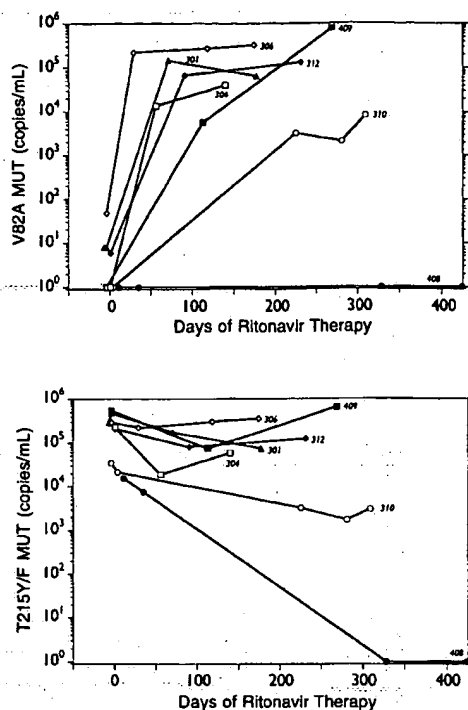


FIG. 3. Impact of development of the V82A protease resistance mutation on the RT T215Y/F ZDV resistance mutation in the indicated subjects.

follow-up. Clearly, with longer follow-up, estimates of the upper limit of s decrease.

Factors affecting the durability of the virologic response. A multifactor one-way analysis of variance of the time to return of the HIV plasma RNA levels towards baseline with respect to the drug dosage, maximal decrease in RNA levels from baseline, baseline RNA levels, nadir RNA levels, and T215Y/F in this small cohort of 10 subjects found only the baseline plasma HIV RNA level ($r = -0.85$; $P = 0.002$), the HIV RNA level at the nadir ($r = -0.88$; $P = 0.0007$), and the drug dosage ($r = 0.96$; $P < 0.00001$) significantly correlated with the time to return to baseline RNA levels (Spearman rank order correlation). Data from 16 additional ritonavir-treated subjects with

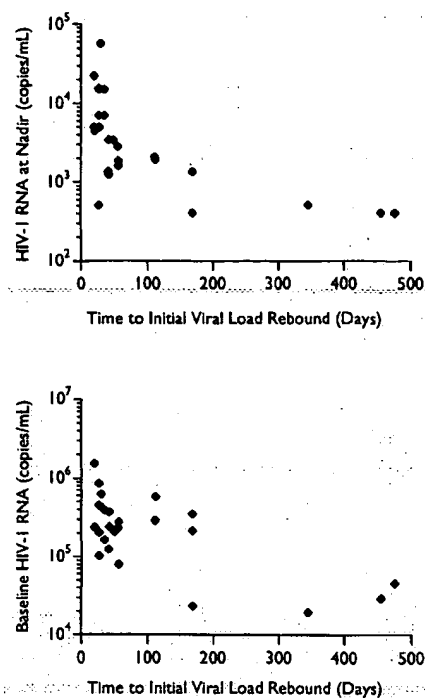


FIG. 4. Impact of baseline and nadir plasma RNA levels on duration of virologic response.

respect to baseline viral RNA levels and drug dosage were included to increase the power, and the statistical analysis was repeated. Baseline RNA levels remained weakly, but significantly, correlated ($r^2 = 0.45$; $P = 0.0001$), as did HIV RNA levels at the nadir ($r^2 = 0.38$; $P = 0.0008$), while drug dosage lost significance ($r^2 = 0.16$; $P = 0.04$) (Pearson product-moment correlation), although area under the curve (AUC) and trough levels of drug concentration (C_{min}) were not available for analysis. The impacts of baseline and nadir RNA levels on the durability of the virologic response are presented in Fig. 4. Inspection of the plot of the HIV RNA levels at the nadir reveals that no subject in this study had a virologic response longer than 60 days unless plasma HIV RNA levels fell below 2,000 copies/ml. Interestingly, both the baseline viral RNA

TABLE 4. Relative fitness of HIV populations containing the protease V82A or RT T215Y/F mutation following drug withdrawal

| Subject | Mutation ^a | Day | s | 95% Confidence limit | | r^2 | Additional genotypes |
|---------|-----------------------|-----|----------------|----------------------|-------|-------|---------------------------------------|
| | | | | Lower | Upper | | |
| 408 | RT T215Y/F | 395 | 0.030 | 0.012 | 0.048 | 0.998 | M41 WT, K70 WT |
| 409 | RT T215Y/F | 311 | 0.039 | -0.006 | 0.083 | 0.992 | M41 WT, K70 WT |
| 312 | RT T215Y/F | 231 | 0.018 | -0.057 | 0.078 | 0.797 | M41L MUT ^c , K70 WT |
| 304 | RT T215Y/F | 140 | 0.021 | -0.101 | 0.120 | 0.549 | M41L MUT , K70 WT |
| 310 | RT T215Y/F | 313 | $\leq 0.019^b$ | | | | M41L MUT , K70 WT |
| 306 | RT T215Y/F | 178 | $\leq 0.033^b$ | | | | M41 WT, K70 WT |
| 301 | RT T215Y/F | 184 | $\leq 0.032^b$ | | | | |
| 401 | PR ^d V82A | 198 | $< 0.029^b$ | | | | I54V MUT, L63P MUT |
| 409 | PR V82A | 42 | $< 0.138^b$ | | | | I54V MUT, L63P MUT |

^a Probe hybridization interrogates a specific mutation; however, this viral population most likely represents virus that is multiply MUT following extended therapy.

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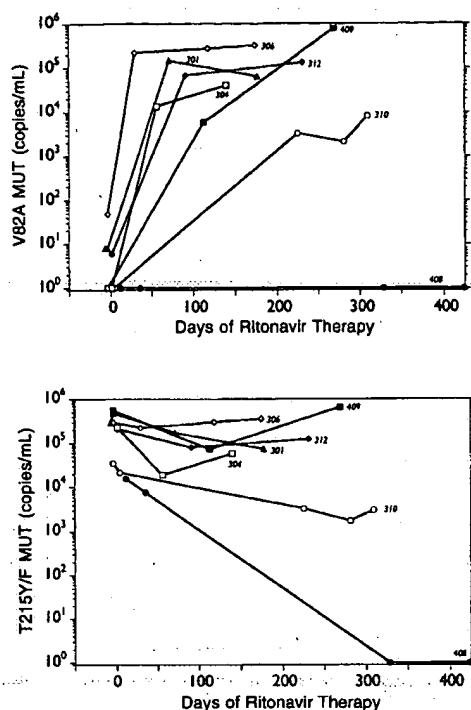


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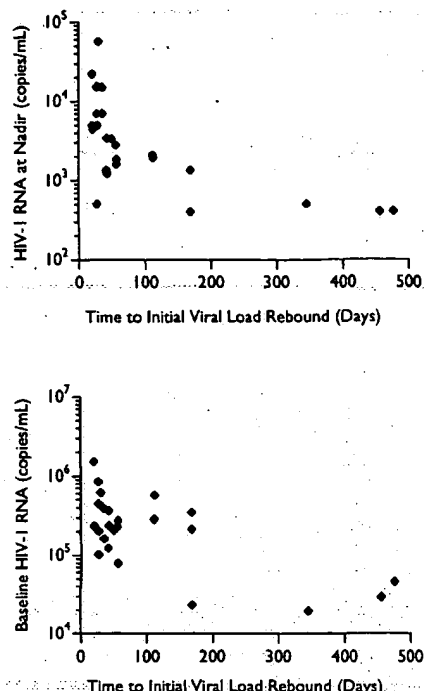


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DISCUSSION

Recent clinical studies demonstrate dramatic decreases in plasma HIV RNA levels and dramatic increases in $CD4^+$ cells with zidovudine monotherapy (8, 26). After extended zidovudine monotherapy, however, plasma virus RNA levels began to rise in many subjects, indicating a loss of susceptibility to the drug. Molla et al. (28) have previously reported on an ordered accumulation of mutations during zidovudine monotherapy based on their frequency of appearance. In order to identify genotypic changes which were associated temporally with rebounds in the plasma HIV RNA levels in vivo, differential hybridization of the RT-PCR product derived from plasma virus RNA (10, 16) was coupled with plasma RNA levels in longitudinal samples from 10 patients. Using this approach, we have been able to define three types of mutations: primary, secondary, and compensatory viability.

Primary resistance mutations were temporally and quantitatively associated with the initial loss of suppression of viral replication. For zidovudine monotherapy, the appearance of the V82A/F mutation was identified as the primary mutation in all eight subjects who experienced viral load rebounds (Fig. 1 and 2) and was not observed in those subjects with a prolonged virologic response. Secondary mutations were associated with subsequent additional losses in viral suppression. In two subjects who had higher doses of zidovudine (304 and 409), a secondary rebound in circulating plasma viral RNA levels was associated with the acquisition of an I54V mutation and conversion of the replicating viral population to I54V-V82A double MUT. The I84V mutation, which was the first change observed with in vitro selection (25), was observed in only one subject, 310, as a secondary resistance mutation.

Two patterns of acquisition of the I54V-V82A double mutation were observed wherein the appearance of I54V was greatly delayed at lower drug doses and was not associated with changes in plasma HIV RNA levels. However, at higher drug doses, the appearance of the I54V secondary mutation was much more temporally related to the appearance of the V82A primary mutation and the return of plasma HIV RNA levels to baseline values. This suggests that at higher drug doses, the V82A MUT alone may require additional genotypic changes, i.e., I54V or I84V, to overcome the selective pressure of the drug.

The coincident expansion of the L63P and V82A populations (Fig. 1 and 2) suggests that L63P may play a role in resistance to zidovudine in vivo. Baseline viral populations from many of these subjects are completely L63P/A or represent mixtures, yet potent and durable virologic response is observed with therapy (e.g., subjects 310, 401, 408, and 409) indicating that this mutation is not a primary resistance mutation. In vitro studies suggest L63P may compensate for changes around the active site, such as at codons 82 and 84 (25). Individual changes at codons 46, 63, and 71 in a HIV-1_{NL4-3} backbone produce phenotypic sensitivities to zidovudine identical to that of WT virus (25). It is possible that the observed changes at codon 63 may restore viability to a replication-challenged V82A/F MUT. Indeed, a lack of viability of virus possessing changes at V82 or V82 and I84 has been reported (25, 34, 38). Changes at codon 10 (38) or 71 (34) have been proposed to compensate for a reduced viability of virus from site-directed mutagenesis stud-

ies in vitro. Furthermore, with purified enzyme preparations, the V82A protease exhibited a reduced enzymatic activity (23, 31, 34), while a secondary A71T change was able to restore some of the enzymatic activity (23, 34). The L63P MUT appeared to improve the in vitro growth kinetics of the V82F-I84V double MUT (25). It is, therefore, possible that a change from the consensus leucine at codon 63 may represent a compensatory viability mutation.

Continued viral replication in the presence of drug resulted in the accumulation of additional genotypic changes. Phenotypic sensitivity of PBMC isolates also demonstrate a progressive loss of susceptibility with continued zidovudine therapy. Two subjects eventually developed the L90M mutation, a primary zidovudine resistance mutation (17, 18), indicating the evolution of cross-resistance to other protease inhibitors. The L90M mutation, however, was not detected until long after the initial viral load rebound and several additional changes had occurred. It is, therefore, possible that a switch in therapy shortly after the initial viral load rebound may prevent the potential for cross-resistance in these subjects.

Several groups have estimated virus turnover in vivo by measuring the decline of plasma virus levels with the initiation of a potent antiretroviral therapy (14, 36, 49). We wished to investigate the in vivo kinetics of expansion of the V82A MUT population. Linear regression analysis of the amount of V82A MUT virus in the plasma during the first-order logarithmic expansion resulted in estimates of 2.4 to 4.8 days (Table 2) for the doubling time of the V82A MUT population. The estimated doubling times most likely represent overestimates and could be improved with more frequent samplings. These doubling times are very similar to those calculated for the Y181C nevirapine resistance mutation (13). Consequently, assuming an average doubling time of 3 days and no delay due to pharmacologic delays, etc., 19 generations, or 57 days, would be required for a resistant mutation with a predrug prevalence of 3×10^{-5} , the mutation rate of HIV (24), to dominate the replicating viral population. Almost identical estimates are obtained with the $CD4$ model.

Coffin has proposed that a single-point mutation occurs between 10^4 and 10^5 times/day in an HIV-infected individual (5). Furthermore, the steady-state level of the MUT should be a function of the forward and backward mutation rates and the selective disadvantage of the mutation to the viability of the virus, of which the latter has the greater impact. We therefore wished to estimate the level of the V82A MUT at the time of initiation of therapy in order to estimate the selective disadvantage of V82A MUT virus in vivo. To this end, we used a mathematical model similar to that of Perelson et al. based on $CD4^+$ T cells as potential target cells (36). The limit of detection of the differential hybridization assay was used to define an upper limit of the amount of V82A MUT virus at the initiation of therapy and, therefore, a maximum fitness. A linear extrapolation during the exponential expansion of the V82A MUT population back to baseline was employed to define a lower limit of the pretreatment levels of resistant virus. This method does not take into account such considerations as a delay in antiviral effects due to the time required for drug absorption, distribution, and penetration into the target cells nor the delay resulting from the mechanism of the protease inhibitor, in which only subsequent rounds of infection are prevented. The estimated s values of the V82A MUTs derived from estimates of the prevalence of the MUT population at the initiation of drug treatment (Table 3) are consistent with the interpretation that these MUTs were present before drug therapy. Under mutation-selection balance, the expected steady-state frequency of a MUT in a host prior to

levels ($r^2 = 0.55$; $P < 0.00001$) and the extent of decrease of RNA from baseline ($r^2 = 0.40$; $P = 0.0006$) were correlated with the level of plasma RNA at the nadir, while drug dose was not significant. Again, AUC and C_{\min} were not available for analysis.

DISCUSSION

Recent clinical studies demonstrate dramatic decreases in plasma HIV RNA levels and dramatic increases in CD4⁺ cells with zidovudine monotherapy (8, 26). After extended zidovudine monotherapy, however, plasma virus RNA levels began to rise in many subjects, indicating a loss of susceptibility to the drug. Molla et al. (28) have previously reported on an ordered accumulation of mutations during zidovudine monotherapy based on their frequency of appearance. In order to identify genotypic changes which were associated temporally with rebounds in the plasma HIV RNA levels in vivo, differential hybridization of the RT-PCR product derived from plasma virus RNA (10, 16) was coupled with plasma RNA levels in longitudinal samples from 10 patients. Using this approach, we have been able to define three types of mutations: primary, secondary, and compensatory viability.

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drug therapy will be roughly μ/s , where μ ($\sim 3 \times 10^{-5}$ [24]) is the mutation rate and s is the selective disadvantage of the resistance mutation in the absence of drugs (5, 29). The estimates for the initial frequency of V82A mutation (Table 2) in the two subjects (306 and 312) fall within the range that one would expect from mutation-selection theory (i.e., $s \sim 0.01$). The somewhat lower predrug frequency of the V82A mutation in subject 301 (whose virus was WT at all amino acids in the protease prior to drug therapy) may reflect the lower fitness of the V82A MUT in the absence of compensatory mutations.

An alternative approach to determining the relative fitness of a resistant viral population *in vivo* is to determine the rate of reversion to WT following drug withdrawal. As part of the protocol, subjects were removed from previous antiretroviral therapies 2 weeks prior to initiation of ritonavir therapy. The removal from ZDV therapy allowed an estimation of the selective disadvantage of the RT T215Y/F mutation. No significant change in the proportion of RT T215Y/F ZDV resistance mutations was observed in those subjects who experienced a rebound in plasma virus RNA levels and rapid development of the V82A mutation in the protease (Fig. 3), indicating the independence of the target genes. The lack of reversion of the RT T215Y/F genotype with ritonavir monotherapy is very similar to the extended periods required for reversion of this phenotype following drug withdrawal (1, 3, 43). So, while dramatic turnover of sequences in the protease gene is observed with ritonavir therapy, the sequences of the RT appear to be quite stable in the absence of any selective pressure on this gene. This is to be expected from a model of quasispecies of two loci under independent selective pressures. Similar results have been reported by Brown and Cleland (4) regarding the independent evolution of the *env* and *pol* genes during ZDV therapy. This suggests that therapy with a protease inhibitor will most likely not be successful in reversing RT inhibitor resistance. Furthermore, the independence of these loci suggests that effective combination therapies directed towards different genetic targets will require multiple, independent genetic changes in a single genome in order to completely escape viral suppression.

Linear regression analysis of the changes in the proportions of the T215Y/F MUT and WT populations following withdrawal from ZDV therapy resulted in estimates of s as ~ 0.02 to 0.04 (Table 4). In three subjects, in whom RT 215 WT sequences could not be detected throughout the course of study, only estimates of the upper limit of s were possible. One disadvantage of this approach is that additional mutations in the replicating viral population not detected by the probe used to interrogate the amino acid in question may increase the fitness of the population. Interestingly, s values from those subjects who harbored virus that was doubly MUT with M41L and T215Y/F appeared to have a slightly greater fitness, suggesting that the M41L mutation may impart additional fitness as well as additional resistance. The advantage of this approach, however, is the *in vivo* nature of the results.

Two subjects with limited follow-up were withdrawn from ritonavir. Plasma virus from these subjects (401 and 409) was found to remain completely V82A MUT at 198 and 42 days, respectively, following withdrawal from drug. An estimate of the upper limit for the selective disadvantage for the V82A mutation relative to WT at codon 82 in the subject with the greatest follow-up, 401, was approximately 0.03. The larger upper limit estimate for the selective disadvantage for the V82A mutation in subject 409 is a reflection of the shorter time following drug withdrawal. The estimates of the fitness of the expanding V82A MUT population, based on the CD4⁺-T-cell model, are in general agreement with these estimates. As de-

scribed above, with continued ritonavir therapy following the viral load rebound, additional mutations accumulate. Indeed, our hybridization data reveals the presence of a triple (I54V-L63P-V82A) MUT population in both of these subjects at the time of drug cessation (Fig. 2). It is therefore possible that the accumulation of additional mutations served to decrease the pleiotropic cost of the V82A mutation in the absence of drug.

The lack of a significant fitness loss in the final population of both ZDV- and ritonavir-resistant MUTs points to a potentially serious future problem with respect to antiviral resistance. It suggests that in patients in whom multiply mutated drug-resistant variants have appeared, the resistant strains will not quickly recede following the withdrawal of therapy. The amount of time for a resistant strain to decrease to a given level will depend critically on the selection coefficient s . For a selective disadvantage of 0.02, the time required for the density of the resistant virus to drop to $<0.01\%$ of its original level in the complete absence of drug will be on the order of 461 days.

Finally, the duration of virologic response to ritonavir monotherapy, as monitored by plasma HIV RNA levels, was correlated with plasma RNA levels at both baseline and the nadir (Fig. 4) and weakly correlated with drug dosage. It is likely that evaluation of AUC or C_{min} may result in a better correlation with duration of therapy. Danner et al. have previously reported a clear relation between increasing dosage and the duration of the response (8). Molla et al. (28) have reported both the AUC and trough levels were more significantly associated with the rate of selection of mutations in the protease during ritonavir therapy than was drug dose, most likely due to intrasubject variation in the pharmacokinetics of ritonavir. Interestingly, Havlir et al. have reported an improved durability of virologic response in some subjects with a higher dosage of nevirapine despite the detection of virus with reduced phenotypic sensitivity to nevirapine (12). Similarly, if drug trough levels can be maintained well above the 99% inhibitory concentration of the V82A/F MUT virus, extended suppression of viral replication may be possible.

While the correlation of plasma HIV RNA levels at the nadir with the time to increase of plasma RNA toward baseline levels was significant ($r^2 = 0.38$; $P = 0.0008$), Fig. 4 illustrates the significance of this correlation. In this study, no subjects in whom plasma virus RNA levels were not suppressed below 2,000 copies/ml experienced a virologic response greater than 60 days. While there were subjects whose plasma virus RNA levels were suppressed below this level that had short durations of viral suppression, this may have been confounded by other factors, such as drug dose and baseline levels of viral RNA. It is interesting to note that this time frame is in good agreement with our estimates of the time required for a preexisting resistance mutation at a prevalence of 3×10^{-4} to 1×10^{-6} to dominate the replicating viral population (see Results), given a doubling time on the order of 3 days.

In conclusion, we have extended the observations of Molla et al. (28) by identifying mutations that were associated with initial rebounds in plasma HIV RNA levels (primary mutations) and mutations that were associated with subsequent additional rebounds in plasma HIV RNA levels (secondary mutations). During ritonavir monotherapy, the V82A/F mutation was identified as a primary mutation while I54V and I84V were identified as secondary mutations. With continued ritonavir therapy, additional genotypic changes were observed and associated with further losses in phenotypic susceptibility. An L90M saquinavir resistance mutation was occasionally observed, which may prevent subsequent therapy with alternative protease inhibitors.

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ranged from 2.4 to 4.8 days. As a consequence, HIV RNA levels frequently returned to baseline in a very short period of time, often weeks. The fitness of the V82A MUT, as well as the RT T215Y/F ZDV-resistant MUT, was estimated to be ~2 to 4% less than that of WT following drug withdrawal, suggesting reversion to WT may not occur rapidly. The duration of the virologic response was correlated with plasma RNA levels at the nadir. No subject with plasma RNA levels >2,000 copies/ml at the nadir experienced a virologic benefit greater than 60 days, consistent with doubling time estimates for complete conversion of the replicating viral population. Complete suppression of viral replication and a long-term virologic response were associated with low baseline viral loads and higher drug levels. These results argue for the early initiation of effective therapy when plasma viremia is low to thoroughly suppress viral replication and prevent the development of drug-resistant variants.

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ranged from 2.4 to 4.8 days. As a consequence, HIV RNA levels frequently returned to baseline in a very short period of time, often weeks. The fitness of the V82A MUT, as well as the RT T215Y/F ZDV-resistant MUT, was estimated to be ~2 to 4% less than that of WT following drug withdrawal, suggesting reversion to WT may not occur rapidly. The duration of the virologic response was correlated with plasma RNA levels at the nadir. No subject with plasma RNA levels >2,000 copies/ml at the nadir experienced a virologic benefit greater than 60 days, consistent with doubling time estimates for complete conversion of the replicating viral population. Complete suppression of viral replication and a long-term virologic response were associated with low baseline viral loads and higher drug levels. These results argue for the early initiation of effective therapy when plasma viremia is low to thoroughly suppress viral replication and prevent the development of drug-resistant variants.

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